THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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Junior Members

David William Allen; David Betteridge, B.Sc., Ph.D. (Birm.).

DEATH

We record with regret the death of

Charles Harcourt Wordsworth.

NORTH OF ENGLAND SECTION

The twenty-fourth Summer Meeting of the Section was held at the Prince of Wales Hotel, Scarborough, from Friday, June 9th, to Monday, June 12th, 1961.

The Chairman of the Section, Mr. J. Markland, B.Sc., F.R.I.C., presided over an Ordinary Meeting at 10.15 a.m. on Saturday, June 10th, at which Dr. H. A. Thomas gave a lecture (illustrated by a sound film) entitled "Automation."

On the Saturday evening, the party saw the show at the Futurist Theatre, Scarborough, and on the Sunday afternoon made a coach tour, taking tea at Ravenscar.

WESTERN AND MIDLANDS SECTION

A joint Summer Meeting of the Western and Midlands Sections was held on Friday and Saturday, May 26th and 27th, 1961, in Hereford.

On the afternoon of Friday, May 26th, a visit was paid to the Cider Factory of Messrs. H. P. Bulmer & Co. Ltd. At 7 p.m. a meeting was held in the Town Hall, at which the Chair was taken by the Chairman of the Western Section, Dr. G. V. James, M.B.E., M.Sc., Ph.D., F.R.I.C. A film on "An Introduction to Ion Exchange" was shown and the following paper was presented: "The Application of Ion Exchange Resins to Metallurgical Analysis," by J. R. Millar, M.A., F.R.I.C.

On Saturday there was a coach tour of the Vale of Evesham and an informal dinner at the Green Dragon Hotel in the evening.

MICROCHEMISTRY GROUP

THE thirtieth London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, June 21st, 1961, at "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Chairman of the Group, Mr. C. Whalley, B.Sc., F.R.I.C.

The subject for discussion was "Quantitative Paper Chromatography," which was opened as follows: "Inorganic," by E. C. Hunt, B.Sc.; "Organic," by D. Gross, Ph.D.

BIOLOGICAL METHODS GROUP

THE Summer Meeting of the Group was held on Friday, June 9th, 1961, and took the form of a visit to Boots Pure Drug Co. Ltd., Nottingham.

In the morning a tour was made of the Pharmaceutical Manufacturing Factories and after lunch a visit was made to the Biological Laboratories.

Twenty-nine members and friends attended and their thanks to the Company were proffered by Mr. J. S. Simpson, F.I.M.L.T., Chairman of the Group.

Coulometric Methods in Analysis

A Review*

BY D. T. LEWIS

(*D.S.I.R., Laboratory of the Government Chemist, Clement's Inn Passage, London, W.C.2*)

Modern applications of the principles of coulometry to analytical problems are reviewed. The types of electrolytic apparatus required for the electrogeneration of reactant materials are described, special attention being paid to the various electrical indicator systems now being employed for titrimetric end-point determinations. Alternative chemical or photometric indicator methods receive general mention.

The recent applications of oxygen and adsorption electrode devices are illustrated; electrolytic hygrometry is discussed, and some examples are given of the use of coulometry for the quantitative analysis of pesticide residues on agricultural crops, the determination of oxide tarnish on metals and the determination of tin-alloy coatings on iron. The principles of the commercial "fuel cells" and their possible analytical applications are briefly indicated.

1. INTRODUCTION

The general electrolytic principles governing the applications of coulometry to analysis have remained unchanged since their enunciation by Michael Faraday in 1833. These fundamental laws may be concisely represented by the equation—

$$w = \epsilon it = \frac{Eit}{F} \qquad (1)$$

where w is the weight in grams of an element formed by a primary electrode process produced by the passage of i amperes of current for a period of t seconds, ϵ is the electrochemical

* Reprints of this paper will be available shortly. For details, please see p. 556.

equivalent and is obviously the weight of the particular element produced by one coulomb, E is the gram equivalent weight of the element and F is the Faraday unit expressed in coulombs.

The application of equation (1) to any analytical problem can only become possible if physical instrumentation is available that can measure in a precise quantitative manner the three connected variables, weight, current and time, or which will give related physical information from which the magnitudes of these variables may be determined. Determinations that can often be made reproducibly to a precision of 0·1 per cent. and an equivalent accuracy will usually satisfy the demands of the most critical analyst, and, if modern techniques of measurement are employed, the coulometric method will invariably satisfy this criterion, not only in the macro range, but in the micro range as well.

2. Chemical and electrical standards

The International System of 1908 defined the ampere as that current which would deposit 0·001118 grams of silver per second in a voltameter of special design. This definition became obsolete in 1948 when the Ninth General Conference of Weights and Measures decided that the more fundamental absolute units were becoming known with a certainty exceeding that attending the older "international units." The theoretical re-definition of the ampere employing Neumann's integral has resulted in the relationship—

$$1 \text{ International ampere} = 0{\cdot}99985 \text{ absolute ampere.}$$

From the analyst's point of view, this change is of insignificant character, although the new value of the coulomb is also affected to a similar extent.

The most recent physical measurements of the basic natural constants have yielded the following values[1]—

(60) Avogadro's Number (Chemical) $= 6{\cdot}02308 \times 10^{23}$ atoms per mole.

(45) Electronic charge $= e = 1{\cdot}60207 \times 10^{-20}$ e.m.u.

(3) Velocity of light $= C = 2{\cdot}997929 \times 10^{10}$ cm per second.

(26) Faraday $= \dfrac{Ne}{C} = 9649{\cdot}4$ e.m.u. per gram equivalent $= 96{,}494$ coulombs per gram equivalent.

The figures in parenthesis preceding the name of each constant give the probable errors in parts per million of the numerical factors. Since the value for e may be expressed as $1{\cdot}60207 \times 10^{-19}$ coulombs, it is obvious that an ampere of current is also associated with the transfer of $6{\cdot}24 \times 10^{18}$ electrons per second.

Silver is employed quite widely in coulometry as an electrogravimetric standard. It is appreciated that it consists of two isotopes of atomic masses 106·950 and 108·949, present in amounts equivalent to 51·9 and 48·1 per cent., respectively. The migration velocities in aqueous solution of these two ionic forms must be exactly equivalent, because there have been no reports of isotopic enrichment during electrolysis.[2] Such enrichment can occur during the electrolysis of fused salts at high temperatures.

Shields, Craig and Diebeler[2] at the U.S. Bureau of Standards, in a study of the absolute abundance ratio of silver to be used for the accurate electrochemical determination of the Faraday, have discussed the various conflicting results for the chemical atomic weight of this element, the 1957 International Value being Ag = 107·88. On the basis of their mass-spectrometric observations, these workers suggest that the chemical atomic weight (O = 16) is more correctly given by $Ag = 107{\cdot}8731 \pm 0{\cdot}0020$, a result differing significantly from the usually accepted value.

The position of silver in the electrochemical series makes it particularly useful as a coulometric standard. If we consider a neutral decimolar solution of silver nitrate of ionic strength 0·1 and of activity coefficient $f_{Ag^+} = 0{\cdot}77$, we have, at 25° C, the following Nernst equation for the reduction $Ag^+ + e = Ag$.

$$E_{Ag} = E^0_{Ag} + 0{\cdot}05915 \log f_{Ag^+} C_{Ag^+} = 0{\cdot}7990 - 0{\cdot}0659 = 0{\cdot}7331 \text{ volt.}$$

At the inert anode, the evolution of oxygen proceeds according to the scheme—

$$2OH^- \rightarrow \tfrac{1}{2}O_2 + H_2O + 2e$$

where $a_{H^+} \times a_{OH^-} = K_W = 10^{-14}$ in neutral solution, *i.e.*

$$E_{O_2} = E^0_{O_2} + 0{\cdot}02957 \log a^2_{OH^-} = -0{\cdot}402 + 0{\cdot}02957 \log (10^{-7})^2 = -0{\cdot}8159.$$

The theoretical reversible decomposition potential of silver nitrate is thus quite small, *i.e.*,

$$E_{rev.} = E_{Ag} + E_{O_2} = 0{\cdot}7331 - 0{\cdot}8159 = -0{\cdot}0828 \text{ volt.}$$

Moreover, if we consider the presence of 0·01 M copper as an impurity in our silver solution, $f_{Cu^{++}} = 0{\cdot}5$ when the ionic strength is 0·03, and the reduction potential of cupric ion is given by—

$$E_{Cu^{++}} = 0{\cdot}3441 + 0{\cdot}02957 \log f_{Cu^{++}}C_{Cu^{++}} = 0{\cdot}3441 - 0{\cdot}0680 = 0{\cdot}2761 \text{ volt.}$$

Neglecting polarisation effects, the concentration of silver would have to fall to about 10^{-9} gram ions per litre before copper impurity became deposited on the silver cathode, *i.e.*, when E_{Ag^+} becomes 0·2761 volt. Even lower concentrations of lead and tin would obviously be tolerated, because of their more positive oxidation potentials, and at a controlled-cathode potential, virtually pure silver is deposited.

3. Measurement

(*a*) Measurement of current and time—

A precision stopwatch is used by many laboratories for the exact measurement of electrolysis time, the main objection to its use being that it is difficult to switch on the current and start the watch with complete simultaneity. For this reason, electrical or electronic timing units are generally preferred. They can form part of the electrolytic circuitry and give the advantage of being under the single switch control that also controls the current. Times accurate to one-hundredth of a second are possible with modern types of electric chronometers.

For the accurate independent measurement of current, the preferred method involves the use of a calibrated resistor incorporated directly into the series circuit of the coulometric cell. The potential drop across the resistor is determined with a precision vernier potentiometer. Such instruments are available in the United Kingdom and will measure from 2 volts to microvolts with an error of 0·002 per cent.

If the electrolysis is carried out at constant current, the total number of coulombs consumed is given by $q = it$ coulombs.

If, however, an electrolysis is carried out at controlled potential, the current will usually decrease logarithmically with time according to the expression—

$$i = i_0 e^{-kt} \qquad (2)$$

where i_0 is the initial current, the total number of coulombs being given theoretically by the integral—

$$q = \int_0^\infty i\,dt = \int_0^\infty i_0 e^{-kt}\,dt = \frac{i_0}{k} \qquad (3)$$

It is obviously impossible to carry out an experiment to infinite time, and in practical analysis it will suffice if the electrolysis is followed graphically until the final observed current i is

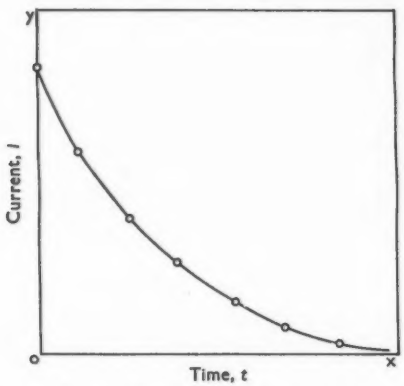


Fig. 1. Plot of current against time

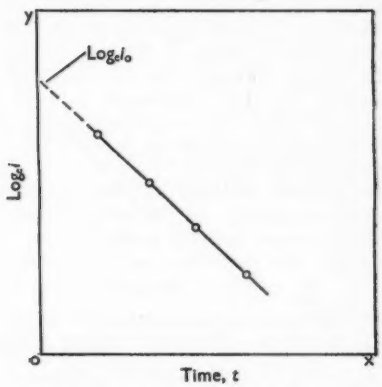


Fig. 2. Plot of $\log_e i = \log_e i_o - kt$

of the order of 0·1 per cent. of the original i_0. This gives a curve of the type shown in Fig. 1, which may be integrated by counting the graphical squares or by cutting out the segment and comparing its weight with a square of the same graph paper of known area. Alternatively, MacNevin and Baker[3] have plotted (Fig. 2) the linear equation $\log_e i = \log_e i_0 - kt$, and the intercept $\log_e i_0$ and the slope, $-k$, are found from the graph. A few points will establish the straight line, and the integral, equation (3), is then readily evaluated. The previous authors employed this method for determining the oxidation of the lower valency states of iron and arsenic by graphically following the coulometric titration over a period of only 10 minutes.

(*b*) DIRECT MEASUREMENT OF A NUMBER OF COULOMBS—

This method employs a standard chemical coulometer in series with the electrolysis cell and, provided the coulometric reaction proceeds with 100 per cent. efficiency, it will give an absolute integrated measurement regardless of any irregularities in the magnitude of the current. Silver and copper coulometers are widely employed, the increase in weight of the cathode being measured.

Lingane[4] describes a thermostatically controlled gas coulometer with potassium sulphate as electrolyte. Correcting for vapour pressure, atmospheric pressure and temperature, 16,811 ml of gas at S.T.P. = 1 Faraday. Many variants of electrolyte formulation are possible in such gas coulometers.[3,5]

Ehlers and Sease[6] have described the construction of a constant-potential coulometer for use in the 0 to 10 coulomb range. Copper is first deposited by the current to be measured from a copper sulphate solution, and the metal is then subjected to a quantitative anodic-stripping process.

Lingane and Small[7] make use of electrolytically generated acid or base to provide a coulometer accurate to 0·1 per cent. at 10 coulombs and to 1 per cent. at 1 coulomb.

In addition to the chemical coulometers, many ingenious electromechanical and electronic devices have been described for determining the "q" function. Thus Booman[8] has developed an electronic potentiostat and integrator circuit with a 10-microsecond response to current changes and usable in the 10 μA to 10 mA range. Bett, Nock and Morris[9] have described a low-inertia integrating motor whose speed of shaft rotation is a linear function of the applied voltage. This sytem has also been studied by Furman and Fenton[10] who show that an empirical relation of simplifying character exists between the motor-calibration factor and the value of the series resistor that controls the voltage across the motor terminals. The rotation of the armature shaft is followed by a simple mechanical counter.

Strip-chart recorders have been used to study coulomb changes, which are followed mechanically by ball-and-disc integrating systems.[11]

Coulometric devices of this nature are not commercially available in the United Kingdom. Potentiostats and automatically controlled cathode electrolysers may, however, be purchased, the cathode potential being pre-set and controlled automatically during the electrolytic deposition. These devices are also useful in polarographic measurements for the preliminary removal of large amounts of interfering ions by coulometric deposition.

4. ANALYTICAL PRINCIPLES

(*a*) PRINCIPLES OF ANALYTICAL COULOMETRY—

In coulometry the electrode processes may obviously be carried out (*a*) at controlled potential or (*b*) at constant current, the voltage being applied from an external source, *e.g.*, an accumulator. Such processes are examples of "external electrolysis." In some unique instances quantitative electrode processes can occur without use of an external battery, *e.g.*, by the short-circuiting through a resistor of a simple chemical cell. The applications in coulometric analyses of such systems are referred to under "Internal Electrolysis" (see Section 7).

(*b*) ELECTROGRAVIMETRIC ANALYSIS—

It is not proposed in this review to deal in any detail with external electrolysis of the electrogravimetric type involving the weighing of cathode deposits. The phenomena of "over-potential" and "concentration polarisation" are important in all applications of coulometry, hydrogen over-potential being greatest at mercury cathodes (0·8 volt) and oxygen

over-potential being most pronounced for the noble metals (0·5 volt). Such phenomena permit the prior deposition of cadmium ($E^0 = 0{\cdot}402$ volt), whose normal deposition would have been expected to follow that of the more reducible hydroxonium ion (H_3O^+). The electrogravimetric deposition of silver has already been discussed in Section 2.

(*c*) COULOMETRIC TITRATIONS AT CONSTANT CURRENT—

Such titrations differ from volumetric titrations in that the titrant is generated electrolytically, the electron being the standard reagent. The accuracy of these titrations is such that Tutundzic[12] has suggested that the coulomb would be a preferable standard in titrimetric analysis, rather than the conventional chemical standards. The coulometric method is particularly useful for studies in the microgram to milligram range and is capable of great accuracy.

Two types of titrations are usually defined. In primary coulometric titrations the substance to be determined reacts directly at the electrode. In secondary coulometric titrations, a reactive intermediate is first generated quantitatively by the electrode process, and this must then react directly with the substance to be determined. It is obviously a *sine qua non* consideration that the coulometric reactions must occur with 100 per cent. current efficiency, and this demands a completely inert electrode system, absence of reducible gases, such as oxygen, and also complete absence of electroactive solvents, impurities, etc. It is common practice to purge the atmosphere of the electrolyte cell system with pure nitrogen or argon.

The stability of gold, platinum and palladium electrodes in strong oxidising solutions has been critically examined by Lee, Adams and Bricker,[13] who conclude that none of these electrodes are truly inert. Various investigations have shown, however, that platinum anodes may be successfully used for the generation of halogen titrants according to the scheme—

$$2\,Cl^- \rightarrow Cl_2 + 2e$$

The classical papers of Szebelledy and Somogyi[14] have described the internal generation of halogen and subsequent oxidation of thiocyanate, hydrazine, sulphite, etc., and this work has been considerably extended by Swift *et al.*[15] Arthur and Donahue[16] have also demonstrated that electrolysis of titanic chloride at a gold cathode will produce titanous ions, which may be employed successfully as a reductimetric titrant. Tutundzic and Mladenovic[17] have similarly used a platinum anode for the generation of permanganate ion in an acidulated manganous sulphate anolyte. It is therefore obvious that noble metal electrodes can be employed under fairly adverse oxidising - reducing conditions.

(*d*) APPARATUS FOR CONSTANT-CURRENT TITRATIONS—

Most titrimetric coulometry is carried out under constant-current conditions, particular attention being paid to the stabilisation of the current supply. A typical circuit is shown in Fig. 3, the switching on of the current being synchronised with the operation of an electric chronometer, although a stopwatch can obviously be used. A chemical coulometer can also replace both the precision resistor and the chronometer if this is preferred.

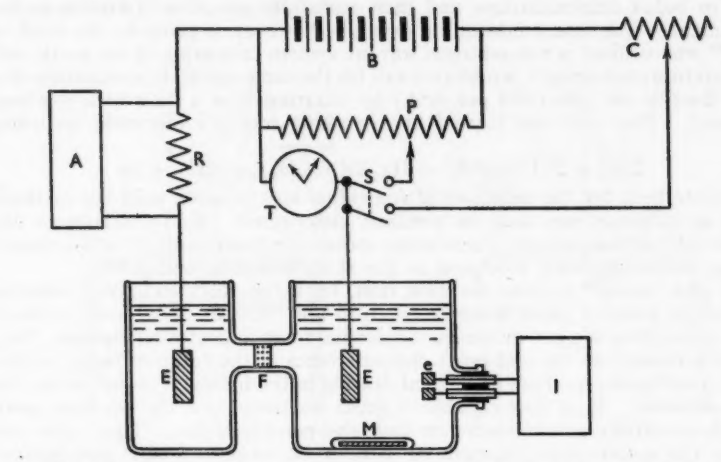
With the circuit shown in Fig. 3, the constant current may be supplied from a 24-volt battery in series with a large resistance or, alternatively, the a.c. mains supply may be employed via a constant-voltage transformer with full-wave rectification and appropriate stabilisation in the output circuit. Currents of 10 to 100 mA would generate from 1×10^{-7} to 1×10^{-6} equivalents of acid, base, metal ion, etc., per second during the electrolysis. The coulometric reaction can occur at either of the generating electrodes, and in general the cathode and anode compartments may have to be separated by a porous frit or agar gel, or both, to form an almost impermeable partition and prevent interaction of the electrode products. The electrolyte in either compartment may be agitated with a mechanical or a magnetic stirrer, and it is an advantage, particularly in controlled-potential coulometry, to keep the rate of stirring as smooth and constant as possible.

The indicator-electrode system shown diagrammatically in Fig. 3 deserves special mention, because it is representative of those special devices employed in coulometry to denote the end-point of a particular titration. Such systems are described in detail in Section 4 (*e*). Sometimes, only one electrode or indicator half-cell is employed, this being coupled potentiometrically with one of the working electrodes.

(*e*) INDICATOR SYSTEMS—

In coulometric acid - base titrations, *e.g.*, the liberation of hydroxyl radical at the segregated cathode of a potassium chloride electrolyte, the normal volumetric indicators, such as phenolphthalein, may be used to observe the end-point. Alternatively, in such instances, one could employ the normal glass-electrode system and find the equivalence point from pH observations. Conductimetric systems also have an obvious application in these instances.

Spectrophotometric methods have also been widely used for end-point indication, the coulometric cell being suitably disposed in relation to a spectrophotometer and the end-point



A = Potentiometer
B = Constant-current supply
C = Rheostat
E = Working electrodes
e = Indicator electrodes or half-cells
F = Permeable partition or frit
I = Indicator meter, e.g., pH meter or microammeter
M = Magnetic stirrer
P = Potentiometric resistor
R = Precision resistor
S = Double-pole switch
T = D.C. or A.C. operated timer

Fig 3. Diagram of coulometric circuit

noted photometrically at that wavelength at which excess of the generated titrant ion absorbs most strongly. For accurate work, measurements of the optical density are made at fixed intervals over the sensitive region of the titration.

As an alternative to these preceding techniques, the so-called "amperometric" indicator-electrode system is much employed. A dry battery impresses through a suitable resistor a constant small potential across the two noble electrodes. The variation in current as recorded on a calibrated microammeter will yield a current - time graph showing marked inflexions in the end-point region. In experiments in the 10- to 100-μg range, the electrolysis occurring at the indicator electrodes must be of negligible order or it will interfere with the accurate determination of the amount of titrant generated at the working electrode.

Leisey[18] has shown that the determination of mercaptans in petroleum oil in alcoholic solution with electrically generated silver ion to precipitate the mercaptide may be fully automated by causing the current increase at the amperometric detector electrodes to energise a relay that stops both the current and an electrical timer.

The widely known "dead-stop" indicator system of Foulk and Bawden has been much used for detecting the Fischer reagent end-point in water determinations. A related indicator system, depending on the rate of change of potential and known as the "derivative polarographic method" has been introduced for redox titrations by Reilley, Cooke and Furman.[19]

Two small platinum or gold electrodes are polarised with a minute constant current. The indicator electrodes show a varying potential difference during the progress of the determination, the rate of change being particularly well marked at the titrimetric end-point. Such a system has been employed by Carson, Vanderwater and Gile[20] in their study of the interferences produced by other substances during the reduction of plutonium^{VI} with electrogenerated ferrous titrant. They used a constant current of 0·1 μA produced by a 3-volt battery in series with a 30-megohm resistor. The value of the polarising current is critical in such applications and requires careful adjustment. Buck, Farrington and Swift[21] have made use of a similar system for the titration of monovalent thallium with bromine.

Potentiometric methods have been widely used for end-point detection in coulometric, acidimetric or redox determinations and their variations are so well known as to need no further comment. One recent interesting example, however, is given by the work of Mather and Anson,[22] who utilised a non-aqueous solvent system consisting of an acetic anhydride - acetic acid mixture containing a perchlorate salt for the coulometric determination of milligram amounts of fluoride ion (error 0·3 per cent.) by titrating it as a base with electrogenerated perchloric acid. This acid was formed at a working mercury electrode according to the scheme—

$$2Hg + 2CH_3COOH \rightarrow Hg_2(OOC.CH_3)_2 + 2H^+ + 2e$$

Most anions interfere, but for solutions of *individual* ions in *acetic* acid the method applies, *e.g.*, nitrate or sulphate ions may be similarly determined. End-points were determined potentiometrically with a mercury - mercurous acetate electrode coupled with a glass electrode, sharp voltage inflexions being produced at the stoicheiometric end-point.

Furman and Adams[23] express the view that, for satisfactory end-point detection in the microgram range, pride of place must be given to the "Sensitive end-point method." This involves the restoration of a pre-determined potential to an indicator cell system, the potential being itself determined by the end-point characteristics of the reaction being studied. This is essentially a null-point method, no current flowing in the indicator circuit when this known potential is achieved. It is thus capable of great accuracy, and the previous workers employed a high-sensitivity galvanometer as their end-point detector. They have applied the technique to the coulometric titration of *p*-methylaminophenol and hydroquinone with electrogenerated cerium^{IV}, the electrolyte consisting of a cerous sulphate solution in 2 M sulphuric acid.

The indicator system was composed of a platinum - iridium anode coupled to a lead - lead amalgam - 2 M sulphuric acid half-cell. The potential of the anode may obviously be adjusted to any value by passing a constant generating current and allowing cerium^{IV} to form at the working electrode in the well stirred solution. The current is passed until the solution potential becomes equal to the indicator-electrode potential and no current flows in the indicator circuit. A known amount of the organic reductant is then added, the solution potential falls and is then again restored to the pre-set potential by electrogenerating ceric ions until zero current is observed in the indicator circuit. The number of coulombs employed to reach this stage can be most accurately determined.

$$Ce^{3+} \rightarrow Ce^{4+} + e$$

5. Controlled-potential methods

If the coulometric electrolysis is carried out at controlled-potential, no indicating-electrode system may be necessary, the magnitude of the final current being sufficient indication of the degree of completion of the reaction.

Shults[24] has employed this method in the indirect determination of plutonium^{VI} with excess of ferrous ion produced at a platinum electrode in sulphuric acid electrolyte, the excess of reductant being determined by coulometric back-titration.

$$4H^+ + PuO_2^{2+} + 2Fe^{2+} \rightarrow Pu^{4+} + 2Fe^{3+} + 2H_2O.$$

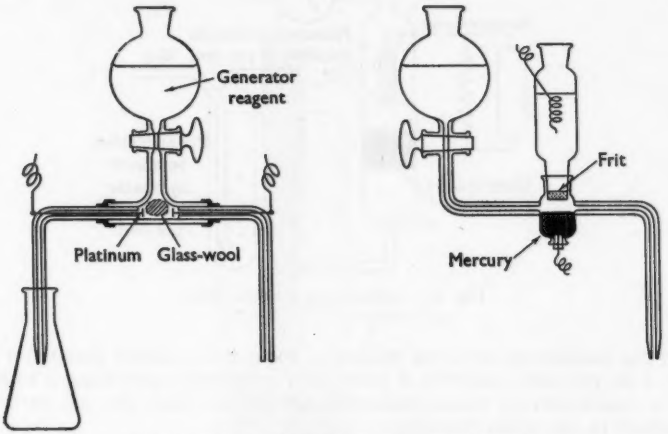
Methods of the controlled-potential type can suffer from the disadvantage of requiring long electrolysis times, but direct indication of the optimum conditions for the successive electrogravimetric deposition of cations can be obtained directly from the polarographic curves for the metal salts.

Lord, O'Neill and Rogers[25] have demonstrated the extreme sensitivity possible by the controlled-potential method and have determined amounts of silver in the millimicrogram range by first electro-depositing the metal and then subjecting it to an anodic stripping

procedure. The current - time curve was automatically produced by a recording potentiometer of known chart speed and the area under the curve assessed with a planimeter (Section 3). A correction is applied for the background-current characteristics of the coulometer.

6. External generation of titrant

One major difficulty attending the internal generation of a titrant in a solution is that other substances or impurities may be present which produce electrolytic interference. These difficulties have been neatly circumvented by Pitts *et al.*[26,27] and Bett, Nock and Morris,[9] who have described external generator cells wherein the titrant is formed externally and allowed to flow via capillary tubes into the solution to be titrated. Typical single-arm and bifurcate assemblies are shown in Figs. 4 and 5.



Figs. 4 and 5. External generators of titrant

Chlorine and bromine may be produced at platinum electrodes in this way and passed into reducing solutions of arsenites, etc. Iodine is said to be best generated in boric acid solution, which neutralises the alkali produced at the cathode.

Reducing titrants, such as ferrous or titanous solutions, may be advantageously produced in this manner from their more highly oxidised forms, and this technique thus eliminates the need for the troublesome bulk storage of these very reactive solutions. Standard quantities of hydroxyl or hydrogen ion may also be coulometrically produced in these devices, which usually incorporate either platinum or mercury electrodes. The method has obvious advantages when the titrant has to be added to hot solutions—

$$2H_2O \rightarrow O_2 + 4H^+ + 4e \text{ (Oxidation).}$$
$$2H_2O + O_2 + 4e \rightarrow 4OH^- \text{ (Reduction).}$$

7. Internal electrolysis

The method of internal electrolysis undoubtedly originated with Ullgren in 1868,[28] who, by short-circuiting the cell shown below for some hours was able to determine the amount of copper in solution with an error of 0·2 per cent. by weighing the platinum dish.

Zinc anode	Sodium chloride (saturated)	Membrane	Copper sulphate solution	Platinum dish cathode

An historical review of this subject has been made by Clarke and Wooten,[29] but the investigatory work recorded in the literature is not extensive. Todt[30] has employed the method to determine minute oxidised films on metal surfaces, the metal being coupled with a cadmium rod in a suitable electrolyte and the current - time characteristics observed. Similar investigations have been made by Oelsen, Graue and Haase.[31]

A most interesting application of the internal electrolysis technique is due to Hersch,[32,33] and this has been elaborated by Keidel[34] into an automatic system for determining trace amounts of oxygen in inert gases. The apparatus consists essentially of the galvanic-cell system shown in Fig. 6.

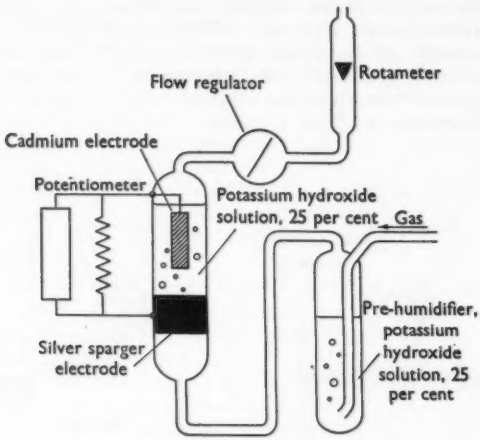


Fig. 6. Coulometric oxygen meter

The inert gas containing parts per million of oxygen is bubbled through a porous silver electrode into a 25 per cent. solution of potassium hydroxide containing a cadmium anode. The oxygen is quantitatively and instantaneously reduced and the cell current generated may be measured in the usual manner—

$$O_2 + 2H_2O + 4e \rightarrow 4OH^- \text{ (Reduction).}$$

$$Cd \rightarrow Cd^{2+} + 2e \text{ (Oxidation).}$$

$$2Cd + O_2 + 2H_2O \rightarrow 2Cd^{2+} + 4OH^- \text{ (Cell reaction).}$$

If one accepts that one Faraday of current reduces 5603·6 ml of oxygen at S.T.P., then, if the flow rate of inert gas containing c p.p.m. of oxygen is f ml per minute, it is readily deducible that the cell current, i, is given by—

$$i = 0{\cdot}287\ f\ c\ P\left(\frac{273}{T}\right)\mu A$$

where P is the pressure in atmospheres and T the temperature in degrees Kelvin of the inert gas under the conditions of the experiment.

With a flow rate of 100 ml per minute and a temperature of 25° C, oxygen impurity equivalent to 1 p.p.m. would theoretically yield a current of 26·7 μA, and this is almost exactly the value obtained in practice.

The method thus offers a most sensitive method for determining oxygen in nitrogen, helium, argon, etc., at levels well below 1 p.p.m., because current magnitudes of unit microampere order are readily measurable. Koyama[35] discusses the application of the Hersch cell system to other gases, *e.g.*, carbon monoxide and carbon dioxide, and replaces the solution of potassium hydroxide in the galvanic cell by potassium hydrogen carbonate. It is obvious that if a de-oxygenated inert carrier gas is used to remove oxygen from liquids, then the Hersch method can also be applied to determinations in liquid media.

Modifications of his original method have also been described by Hersch[36] to determine traces of hydrogen in carrier gas. The gas stream is diluted with a known amount of pure oxygen and allowed to react at 500° C on silica wool, the excess of oxygen being measured galvanically. Other applications, *e.g.*, studies of the corrosion of metals by acids, are referred to in the same paper.

8. Absorption coulometry

Voorhies and Davis[37] have recently described a constant-current coulometric method that involves the initial quantitative adsorption of the electroactive species to be determined on to a layer of compressed acetylene black. This adsorbent then forms one of the working electrodes of a generator cell in which the reducible adsorbed substances are subjected to a cathodic reduction process. Milligram amounts of anthraquinone and cupric ion have been determined in this way after adsorption from solutions.

Acetylene black may be obtained in a state of high purity, it possesses a low redox blank and exhibits a high over-voltage property for both hydrogen and oxygen. Moreover,

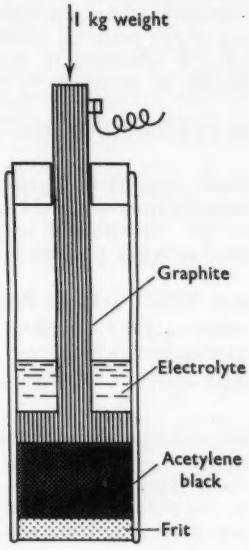


Fig. 7. Adsorption electrode

its contamination by adsorbed atmospheric oxygen is considered of negligible order. It is probable that organic impurities in gaseous systems may be isolated from the gas phase and determined in this manner.

The general design of the working acetylene black electrode is shown in Fig. 7.

9. Coulometric hygrometry

The electrolysis of water in the presence of any suitable acidic electrolyte proceeds in accordance with Faraday's law, oxygen and hydrogen gases being produced, following the consumption of 2 Faradays, according to the scheme—

$$2H^+ + 2OH^- \rightarrow H_2(g) + \tfrac{1}{2}O_2(g) + H_2O \text{ (liq.)}.$$

Since the dissociation constant for water, $k_w = 10^{-14}$, the thermodynamic equation for the reversible decomposition potential at 25° C of an electrolysis cell giving these gases is—

$$E = E^0_{O_2} + \frac{0{\cdot}059}{2} \log k_w^2 = -0{\cdot}403 - 0{\cdot}826 = -1{\cdot}229 \text{ volts}.$$

In practice, because of polarisation effects, the decomposition potential as measured experimentally is usually about 0·5 volt higher for molar solutions. Wexler and Keidel[38] have developed an hygrometer based on the principle that, when a water vapour - air mixture is contacted with solid phosphorus pentoxide in an electrolysis cell composed of the inert plastic Teflon, the wet solid becomes electrically conducting. The pentoxide film is contacted with two spirally wound platinum-wire electrodes, and the absorbed water is quantitatively decomposed by a measured current. The magnitude of this resultant electrolysis current permits the determination of water vapour in the p.p.m. range in air.

At a flow rate of 100 ml per minute of air through the Teflon cell under normal room conditions, 1 p.p.m. of water vapour produces an electrolysis current of about 13 microamperes. The instrument responds in a few minutes to humidity changes of the order of 50 to 100 per cent. of the original value.

10. Coulometric applications of particular interest

(*a*) The extent to which foodstuffs are contaminated by residues of toxic agricultural pesticides is a matter of world-wide interest, and in some countries, *e.g.*, the U.S.A., legislation restricts these residue levels to the p.p.m. range. Normal methods of analysis are time-consuming, but Coulson *et al.*[39] have recently developed a method for determining BHC, aldrin, dieldrin, DDT and other chlorinated organic pesticides in 1 hour. This method depends on a gas-chromatographic separation of the pesticide followed by a combustion, the effluent gases yielding chloride ions, which are absorbed in water and coulometrically titrated with silver. The method is applicable to mixtures of chlorinated pesticides and to the thiophosphates.

Schwabe and Seidel[40] have also examined the direct determination of gamma-BHC by controlled electrolysis.

(*b*) Reilley and Porterfield[41] have employed the electrogenerated complex ion of the soluble mercuric salt of ethylenediaminetetra-acetic acid to titrate milligram concentrations of calcium, lead, zinc, manganese, etc., in strongly ammoniacal solution. The reaction sequence is as follows, the complex ion being produced by reduction at a large mercury cathode:

$$2e + HgE^{2-} \rightarrow Hg + E^{4-}$$
$$Ca^{2+} + E^{4-} \rightarrow CaE^{2-}$$

A pre-titration is necessary to find the blank value due to contaminating ions, the end-points being observed with a potentiometric system. The method is undoubtedly applicable to microgram amounts also.

(*c*) Devanathan and Fernando[42] describe an unusual coulometric method wherein a multivibrator circuit gives constant pulses of electrolysing current to a titration cell, these pulses being accurately counted via a relay operating a Post Office mechanical counter. Minute amounts of reagent may be quantitatively produced in this way, and the method is considered superior to the constant-current method when the amounts to be determined are of the order of 10^{-4} g or less.

(*d*) Wilson and others[43] have employed the method of controlled-potential reduction to study the number, n, of electrons involved in the reduction stages of the aminoacridines. They make use of a time factor, $t_{\frac{1}{2}}$, determined graphically, which represents the time for the initial current to fall to half its original value. The total number of coulombs passed during the reduction is given by

$$q = \frac{i_0 t_{\frac{1}{2}}}{0{\cdot}693} \text{ and } n = \frac{qM}{Fw}$$

where w is the weight or reducible compound of molecular weight M.

(*e*) Kunze and Willey[44] have examined the anodic dissolution of tin and tin alloy, $FeSn_2$, from tinplate anodes immersed in a decinormal hydrochloric acid electrolyte, a graphite rod being used as cathode. A high-speed recording potentiometer was used to determine the potential differences between the anode specimen and a silver - silver chloride indicator electrode. Steps in the potential - time curve correspond to the dissolution of pure tin and tin alloy.

(*f*) Campbell and Thomas[45] have employed the coulometric method to determine T, the thickness of oxide tarnish (in Ångstrom units) on a metal film reduced slowly with a small measured current. The cathode potential rises sharply to the hydrogen discharge potential when reduction is complete. The following equation applies—

$$T = \frac{10^5 \, itM}{A \, nFd}$$

where i is expressed in milliamperes, t in seconds, A is the film area in sq. cm, d is the density of the film and M is the molecular weight of the oxide composing the film.

Similar methods have been described by the Tin Research Institute[46] for the determination of the relative amounts of stannous and stannic oxides on a tinplate surface.

(*g*) Gierst and Mechelynck[47] have made a unique mathematical and experimental study of constant-current coulometry as applied to unstirred, homogeneous solutions and claim that certain determinations can be carried out in times of less than 1 minute with a precision of the order of 0·2 per cent. An equation is developed, based on the laws of electrolysis and of ionic diffusion, which relates the extent of the electrode reaction to a function called "transition time," which is indicative of the time interval between the establishment of a capacity charge on the stationary electrode and the fall to zero of the concentration of electroactive substance in its vicinity. A detailed schematic diagram of the electronic "transitometer" coulometer is given by the authors.

(*h*) Considerable international commercial interest is being shown in the development of "fuel cells,"[48] which are essentially coulometric devices for the direct conversion of chemical into electrical energy with consequent improvement in the thermodynamic efficiency. Typical of these devices is the Sondes Place cell, which operates at 500° to 700° C and has porous electrodes of zinc oxide and metallic silver, which are contacted with fused carbonate electrolyte. These electrodes, when contacted with air or oxygen and combustible sludge gas, respectively, produce electrical current according to the scheme—

$$\text{Air electrode:}\quad O_2 + 2CO_2 + 4e \rightarrow 2CO_3^{2-}$$

$$\text{Fuel electrode:}\quad 2CO_3^{2-} + \tfrac{1}{2}\,CH_4 \rightarrow \frac{5}{2}CO_2 + H_2O + 4e$$

The corresponding Bacon cell utilises porous nickel electrodes and employs hydrogen as fuel; it operates at a temperature of 200° C and at a pressure of 400 lb per sq. inch.[49]

These fuel cells suggest an application of coulometry that has not yet been fully explored in the analytical field. There are obviously possibilities of utilising such systems for determining combustible impurities in inert gas phases, *e.g.*, as laboratory indicator cells for gas-chromatographic effluent gases. It is agreed that for such purposes it would be advantageous to develop a fuel cell that would operate satisfactorily at room temperature, but some cells are already known that can function at approximately 100° C, and it may be possible by employing catalytically active porous electrodes to lower this temperature yet further.

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Determination of Sodium, Potassium and Phosphorus in Biological Material by Radioactivation

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Neutron-activation analysis has been applied to the determination of sodium, potassium and phosphorus in biological material. When a flux of 10^{12} neutrons per sq. cm per second for activation and an anti-coincidence counting unit were used, the ultimate limits of sensitivity for the three elements were approximately 10^{-10}, 10^{-9} and 10^{-10} g, respectively. Radiochemical separation procedures were used, and it was possible to analyse eight samples for all three elements in an 8-hour working day.

When biological material is exposed to thermal neutrons, a large percentage of the induced radioactivity is produced by four nuclides, *viz.*, sodium-24, phosphorus-32, chlorine-38 and potassium-42; the half-lives and characteristic radiations of these nuclides are shown in Table I. The only other nuclide frequently contributing a major proportion of the induced

Table I

Half-lives and radiations of ^{24}Na, ^{32}P, ^{38}Cl and ^{42}K

Nuclide	Half-life	Maximum beta energy, MeV	Gamma energy, MeV
Sodium-24	15 hours	1·39	1·37 and 2·76
Phosphorus-32	14 days	1·71	—
Chlorine-38	35 minutes	4·81	1·60 and 2·15
Potassium-42	12·4 hours	3·60	1·53

activity is manganese-56 (half-life 2·6 hours), the gamma spectrum of which can often be observed after plant material has been exposed to neutrons. The gamma spectra of activated

tomato seeds at three different times after activation are shown in Fig. 1, in which the decay of the peak caused by manganese-56 at 0·85 MeV can clearly be seen; the peaks caused by sodium-24 and potassium-42 are also prominent.

Several methods are available for determining the relative contributions of these nuclides to the total activity. Keynes and Lewis[1] and Reiffel and Stone[2] have utilised simple forms of beta spectroscopy. Chlorine-38 was allowed to decay away, and the hard β-rays from the potassium-42 were determined by counting through a filter sufficiently thick to eliminate the softer β-rays from the other nuclides. Phosphorus-32 was then determined as the residual beta-activity after decay for 1 week, and sodium-24 was found by difference. Gamma spectroscopy[3,4,5] is another possible method of analysis, but, unfortunately, it cannot be used

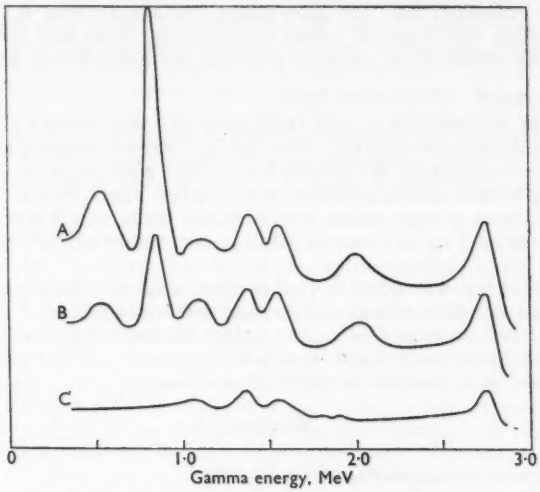

Fig. 1. Gamma spectra of activated tomato seeds: curve A, 3·5 hours after activation; curve B, 7·5 hours after activation; curve C, 25 hours after activation

to measure phosphorus-32 (a pure beta-emitter), and the main gamma energies of sodium-24 and potassium-42 are so close together that they are not well resolved by most gamma spectrometers. A third technique, used by Salmon,[6] involves radiochemical separation of the three nuclides after the addition of carriers. We have used this principle in the work described here; it requires more time and effort than do the other techniques, but is necessary to attain maximum accuracy and sensitivity. It is therefore suitable for analysing small samples of biological material, but offers no obvious advantages when large samples are available. A rapid radiochemical separation of chlorine-38 has been described elsewhere[7] and will not be discussed here.

Experimental

Irradiation—

Liquid standards were sealed into 6-cm lengths of polythene tubing (0·5 mm bore), and samples of seeds were sealed in polythene film and then packed in polythene bags in 3-inch × 1-inch aluminium cans. (Before it was filled, the polythene tubing was washed with 6 N hydrochloric acid and then with water distilled from quartz apparatus; it was subsequently handled with clean Perspex forceps). Each can was irradiated for 15 hours in a flux of about 10^{12} thermal neutrons per sq. cm per second in the Harwell reactor BEPO. This period of irradiation activates about half the theoretical maximum number of sodium and potassium atoms, but only 3 per cent. of the corresponding number of phosphorus atoms. In order to attain higher sensitivity for phosphorus, activation should be for about 14 days, and all samples should then be sealed in silica rather than in polythene, which degrades after several days inside the reactor.

STANDARDS—

The standards used consisted of 0·005-ml aliquots of a solution containing 0·5 μg of sodium and 10 μg each of potassium and phosphorus. The standards were prepared by dissolving the calculated amounts of Specpure sodium hydrogen carbonate and potassium carbonate and analytical-reagent grade ammonium dihydrogen orthophosphate in water distilled from quartz apparatus; they were stored in polythene bottles. Self-shielding during activation was negligible.

Some standards were also prepared by absorbing aliquots of the solution in small squares of Whatman No. 541 filter-paper. This technique was satisfactory for amounts of potassium and phosphorus down to 10^{-7} g, but was useless for sodium because of the amount of this element in the paper. Attempts to wash this residual sodium out of the paper were unsuccessful. It was estimated that the filter-paper contained about 0·35 μg of sodium per sq. cm, which is within the range of values measured by Born and Stark.[8] Possibly, the sodium-24 found was produced by an (n,α) reaction from aluminium present in the paper.

PRELIMINARY TREATMENT AFTER ACTIVATION—

After irradiation, the exterior of each polythene tube was washed with 6 N hydrochloric acid containing sodium as carrier and then with distilled water to remove active contaminants; if this precaution was neglected, the results for sodium were often erratic. The tube was then cut open at each end, and its contents were washed into a 50-ml centrifuge tube with water from a hypodermic syringe, about 2 ml of water being ample for this purpose. Each centrifuge tube contained 1 ml of a carrier solution containing sodium, potassium and phosphorus.

Biological material was wet ashed in 1 ml of nitric acid on a sand-bath at 180° to 200° C, 1 ml of sulphuric acid was then added, and heating was continued for 1 hour, by which time fumes of sulphur trioxide were visible. (Any active halogens were expelled during the wet ashing, which was therefore carried out in a fume cupboard.) The tubes were then cooled to room temperature, and chemical separation was begun.

METHOD

REAGENTS—

All reagents were of recognised analytical grade.

Sulphuric acid, 36 N.

Nitric acid, 16 N.

Hydrochloric acid, 12 N.

Perchloric acid, 70 *per cent.*

Ammonia solution, 15 N.

Magnesium chloride solution, 50 *per cent. w/v*—Prepared from magnesium chloride hexahydrate.

Barium chloride solution, 30 *per cent. w/v*—Prepared from barium chloride dihydrate.

Ammonium carbonate solution, 10 *per cent. w/v.*

Sulphuric acid, 5 *per cent. w/v, in diethyl ether.*

n-*Butyl alcohol*—Saturated with hydrogen chloride gas.

n-*Butyl alcohol - ethyl acetate mixture* (1 + 1 *v/v*).

Wash solution—A solution 6 N in hydrochloric acid and 1 N in sodium chloride.

Combined carrier solution—Prepared by dissolving 37·1304 g of ammonium dihydrogen orthophosphate, 44·5652 g of potassium sulphate and 61·7689 g of anhydrous sodium sulphate in distilled water and diluting to 1 litre.

1 ml ≡ 20 mg each of sodium and potassium and 10 mg of phosphorus.

Potassium carrier solution—Prepared by dissolving 38·1381 g of potassium chloride in distilled water and diluting to 1 litre.

1 ml ≡ 20 mg of potassium.

Sodium carrier solution—Prepared by dissolving 25·4164 g of sodium chloride in distilled water and diluting to 1 litre.

1 ml ≡ 10 mg of sodium.

Combined standard solution—Prepared by dissolving 7·4261 g of ammonium dihydrogen orthophosphate, 3·5345 g of Specpure potassium carbonate and 0·3653 g of Specpure sodium

hydrogen carbonate in water distilled from quartz apparatus and diluting to 1 litre in polythene apparatus.

0·005 ml ≡ 10 μg each of phosphorus and potassium and 0·5 μg of sodium.

SEPARATION OF PHOSPHORUS—

The separation of phosphorus was based on the solubility of dry orthophosphoric acid in diethyl ether[9]; salts of alkali metals are insoluble in ether. The phosphate was purified and then finally precipitated as ammonium magnesium phosphate.

A 15-ml portion of diethyl ether, previously dried over calcium chloride, was added to the contents of each centrifuge tube, and the tube was spun in a centrifuge to coagulate the metal sulphates. The supernatant ether was transferred to a clean tube, and the precipitate was washed twice with 4 ml of ether containing 5 per cent. of sulphuric acid. The precipitate was retained for determining the alkali metals, and the supernatant ether and washings were evaporated by means of a current of air at room temperature. The pH of the residual acid was brought to 9 by adding ammonia solution, and, when cool, 1 ml of magnesium chloride solution was added; the solution was then diluted to 10 ml and swirled. After 30 minutes in a water bath at 20° C, the precipitate was separated by centrifugation and washed three times with water. Finally, it was transferred, as a slurry with acetone, to a weighed aluminium counting tray, dried, weighed and counted; the chemical yield averaged 90 per cent.

SEPARATION OF POTASSIUM—

This separation of potassium was based on the insolubility of its perchlorate, so that the sulphates present had first to be removed.

The precipitate from the extraction with ether was dissolved in water, 2·5 ml of barium chloride solution were added, and the volume was made up to 10 ml. After the tube had been set aside for 10 minutes in a bath of boiling water, the precipitated barium sulphate was separated by centrifugation and rejected, and the supernatant liquid was poured into another tube containing 5 ml of perchloric acid. After being cooled for 15 minutes in a bath of ice, the precipitated potassium perchlorate was separated by centrifugation, and the supernatant liquid was reserved for determining sodium. The potassium perchlorate was freed from traces of sodium by recrystallisation, 0·1 ml of sodium carrier solution and 1 ml of perchloric acid were added to it, and the solution was diluted to 10 ml with water. The precipitate was dissolved by heating at 90° C for 10 minutes and was then re-precipitated by cooling at 0° C for 20 minutes. It was then separated by centrifugation and washed three times with the *n*-butyl alcohol - ethyl acetate mixture. Finally, it was transferred, as a slurry with ether, to a weighed aluminium counting tray, dried, weighed and counted; the mean chemical yield was 40 per cent.

SEPARATION OF SODIUM—

This separation was based on the insolubility of sodium chloride in *n*-butyl alcohol saturated with hydrogen chloride; it was rendered somewhat tedious by the necessity for removing traces of active potassium and inactive barium left from the previous steps.

A 1-ml portion of potassium carrier solution was added to the supernatant liquid from the first precipitation of potassium perchlorate, and the solution was evaporated to dryness in a 100-ml beaker on a hot-plate at 150° C. When cool, the sodium perchlorate was dissolved by boiling with 15 ml of hot *n*-butyl alcohol, the solution was cooled, and the residue of potassium perchlorate was separated by centrifugation. The supernatant liquid was poured into a fresh tube containing 5 ml of *n*-butyl alcohol saturated with hydrogen chloride and was kept at 100° C for 10 minutes. The precipitated sodium chloride was separated by centrifugation, and the supernatant liquid was rejected. The sodium chloride was dissolved in 2 ml of water, 10 ml of ammonium carbonate solution were added, and the solution was boiled for a further 10 minutes. Some barium carbonate was precipitated at this stage, and this was separated by centrifugation. The remaining solution was transferred to a 100-ml beaker, acidified with 0·5 ml of hydrochloric acid, covered with a watch-glass and evaporated to dryness on a hot-plate. Ammonium chloride was removed by heating strongly with a bunsen burner for 5 minutes, and the residue of sodium chloride was cooled, transferred, as a slurry with acetone, to a weighed aluminium counting tray, dried, weighed and counted.

DETERMINATION OF RADIOACTIVITY—

The beta-activities of the precipitates were counted with either a 2B2 end-window Geiger counter (efficiency, about 40 per cent.; background, 30 counts per minute) or an anti-coincidence counter (efficiency, about 10 per cent.; background, 1·5 counts per minute). The low-background counter was suitable only for low count rates and was not often used. Radiochemical purity was checked by counting at intervals for several half-lives (and by gamma spectrometry for phosphorus). The entire procedure, including counting, could be carried out on eight samples by a single individual in 8 hours.

DISCUSSION OF THE METHOD

COMPARISON WITH OTHER TECHNIQUES—

The method was compared with a flame-photometric technique for sodium and potassium and with the molybdophosphate colorimetric technique for phosphorus; these techniques have been described elsewhere.[10] Three replicate determinations were made by each technique, and, as shown in Table II, good agreement was found.

TABLE II

COMPARISON BETWEEN RESULTS BY ACTIVATION AND OTHER TECHNIQUES

Element	Amount of element present, μg	Amount of element found by— activation, μg	Amount of element found by— conventional technique, μg
Sodium	0·0	0·0006	<0·012
	0·1	0·112	0·088
	0·2	0·193	0·202
	0·4	0·399	0·428
	0·6	0·620	0·587
Potassium	0	0·0035	<0·013
	2	2·12	1·92
	4	4·32	4·23
	8	8·01	8·13
	10	9·80	9·97
Phosphorus	0	0·01	<0·15
	2	2·14	2·06
	4	4·20	4·00
	8	8·21	7·87
	10	10·7	10·1

SENSITIVITY AND ACCURACY—

When 0·1-μg portions of sodium, potassium and phosphorus were activated for 15 hours, the respective count rates were approximately 3500, 250 and 200 counts per minute on the 2B2 counter or 1000, 80 and 50 counts per minute on the anti-coincidence counter. These figures are practical and allow for the low chemical yields in precipitating the alkali metals and for decay during the separation. The minimum detectable amounts of the three elements (sufficient to double the background of the anti-coincidence counter) were therefore 0·086 mμg of sodium, 1·2 mμg of potassium and 1·5 mμg of phosphorus. It is possible to attain such sensitivity for sodium and potassium by flame photometry, although not with the simple instrument used by us. However, it is doubtful if any colorimetric method for phosphorus is so sensitive, and the limit can be lowered by a factor of 15 by increasing the period of activation to 1 week.

In practice, it is seldom required to work in the millimicrogram range with these common elements, and the sensitivity is further limited by the magnitude of the blank value. Even after thorough washing, the clean polythene tubes yielded about 0·6 mμg of sodium, 3·5 mμg of potassium and 10 mμg of phosphorus, and for work of the highest sensitivity this blank correction would have to be decreased.

The accuracy of the technique is determined partly by the homogeneity of the neutron flux (±2 per cent. in BEPO) and by errors in weighing the final precipitates and in the transference of standards by pipette; counting errors can be decreased to 1 per cent. by counting for 10,000 counts. The total error is probably less than ±5 per cent.

TESTING THE RADIOCHEMICAL PROCEDURES—

The chemical procedures described above were tested with aliquots of radiochemically pure sodium-24, phosphorus-32, sulphur-35, potassium-42 and manganese-54. Sulphur is a major element in biological material, although it is not activated to a great extent, whereas manganese is much less abundant, but has a very high cross-section for thermal neutrons.[11] The results of the tests are shown in Table III, from which it can be seen that contamination is so small as to be negligible in practice. Chlorine-38 was not used in this test because it is volatilised in the preliminary ashing step and because it has decayed almost completely after 8 hours. Rubidium-86 was not tested either; it would be expected to follow potassium closely, but under our conditions it should not contribute more than 0·2 per cent. to the measured activity for potassium.

TABLE III

CONTAMINATION OF PRECIPITATES BY VARIOUS ELEMENTS

Nuclide tested	Content of nuclide found in precipitate of— sodium chloride, %	potassium perchlorate, %	ammonium magnesium phosphate, %
Sodium-24	40	<0·14	<0·07
Phosphorus-32	<0·05	<0·05	90
Sulphur-35	—	—	1·0
Potassium-42	<0·04	40	<0·07
Manganese-54	0·27	0·11	0·35

INTERFERING NUCLEAR REACTIONS—

Several nuclear reactions could interfere with the determinations described above, but all could be eliminated by carrying out the activation in a thermal-neutron column having no fast-neutron contaminants. Fast neutrons can give rise to interference from the reactions listed below—

(*i*) $^{24}Mg\ (n,p)\ ^{24}Na$
(*ii*) $^{27}Al\ (n,\alpha)\ ^{24}Na$
(*iii*) $^{32}S\ (n,p)\ ^{32}P$
(*iv*) $^{35}Cl\ (n,\alpha)\ ^{32}P$
(*v*) $^{42}Ca\ (n,p)\ ^{42}K$
(*vi*) $^{45}Sc\ (n,\alpha)\ ^{42}K$

Reactions (*ii*) and (*vi*) were of no importance in this work, as the amounts of aluminium and scandium present in biological material are extremely low. The cross-sections for the rest of the reactions are not well known, but appear to be greatest for reactions (*i*) and (*iii*). The magnitude of the interference can only be calculated for specific samples in which the contents of the interfering elements are known. For example, the seeds studied by us contained 0·6 mg of sulphur per g. This would give rise to an amount of phosphorus-32 corresponding to 0·033 mg of phosphorus per g of the original seeds, which is only 0·4 per cent. of the observed value. The magnesium content of the seeds was 0·3 mg per g, but when 0·3 mg of Specpure magnesium was activated, it yielded an amount of sodium-24 corresponding to 0·42 μg of sodium per g of seeds (0·2 per cent. of the observed value). We conclude that these interferences are negligible.

RESULTS—

Tomato seeds were collected under clean conditions from glasshouse plants grown in sand culture on Long Ashton complete nutrient solution. They were found to contain, per gram, 0·19 mg of sodium, 6·96 mg of potassium and 7·75 mg of phosphorus. These results are the means of four replicate determinations and are similar to the values 0·21, 7·0 and 7·0 mg, respectively, found by other methods.[10]

CONCLUSIONS

The neutron-activation method described is a remarkably sensitive technique for determining sodium, potassium and phosphorus. It should be particularly useful for determining traces of phosphorus in biological material, as flame photometry is already established as

a satisfactory method for determining the alkali metals on the micro scale. As far as the biologist is concerned, however, the method is not sufficiently sensitive for the determination of these elements in most individual cells or parts of cells. For example, a single yeast cells weighs 5×10^{-10} g, and its potassium content is of the order of 5×10^{-12} g. Even if fluxes of 10^{14} neutrons per sq. cm per second were used for activation, this amount of potassium would not be detectable, much less determinable. It is therefore evident that the analytical challenge of biological research is not being fully met.

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Rapid Micro-determination of Nitrogen in Fluorine-containing Compounds

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A modified Dumas combustion train for the rapid determination of nitrogen in fluorine-containing compounds and those difficult to combust is described. The tube does not contain a temporary filling, and hydrogen peroxide is used as an intermittent source of oxygen.

The conventional Dumas method for determining nitrogen in organic compounds[1] requires considerable modification when analysing compounds that contain fluorine[2] or are difficult to burn, leaving nitrogenous chars,[3] or when attempting to combust rapidly.[4] With fluorine-containing compounds, tetrafluoromethane[5] often appears to cause the difficulty, and it is possible that CF_3 radicals formed by initial C–C cleavage have time to react with each other instead of with copper oxide, so forming hexafluoroethane. This suggests that the normal pyrolysis temperature of the Dumas train, 600° to 700° C, is too low. The formation of fluorocarbons, which are not absorbed by the potassium hydroxide solution in the nitrometer, will cause high results for nitrogen. According to Sidgwick,[6] the decomposition of hexafluoroethane vapour begins at temperatures in excess of 800° C, so that the first requirement when dealing with fluorine-containing compounds would be appreciably to raise the temperature of the ordinary Dumas train.[7]

A satisfactory technique has been developed in the Microanalytical Laboratory at Cambridge (personal communication from Professor R. N. Haszeldine) in which are used a gas-heated combustion train and a silica tube about 70 cm long containing a permanent filling of copper oxide and copper and, in the "beak" end, a layer of sodium fluoride for absorbing silicon tetrafluoride; the sample is contained in a platinum boat.

The replacement of the copper oxide - copper mixture with nickel oxide permitted Kirsten[8] to use a much higher pyrolysis temperature, 1050° C; although this method and its

modification by Belcher and Macdonald[9] proved to be successful for determining nitrogen in fluorine-containing compounds and those difficult to combust, its disadvantage is high wear of the silica tube, nickel oxide apparently catalysing the crystallisation of silica.

For substances that are difficult to burn and leave nitrogenous chars, so causing low results for nitrogen, many remedies have been suggested, based on a combined pyrolytic and oxidative attack[10,11] as well as on Kirsten's method. Special reference must be made to techniques devised by Unterzaucher,[12] involving oxygen generated by the catalytic decomposition of hydrogen peroxide, Swift and Morton,[3] who used oxygen from a cylinder, and Cropper, Reed and Rothwell,[13] who generated oxygen electrolytically in small amounts at a known rate.

Finally, rapid methods for determining nitrogen based on modifications of the Dumas method have been advocated by many investigators, these modifications involving, for example, increase in temperature[14] and in length of tube,[4,15] high temperature combined with oxygen injection[16,17] and the use of a pre-combustion technique[18] and a specially designed nitrometer.[19]

By increasing the length of the tube to 100 cm and using two high- and one medium-temperature electrically heated furnaces for that part of the tube filled with copper oxide and copper and by inserting hydrogen peroxide as an intermittent source of oxygen in the path of the carbon dioxide, we have been able to combust the classes of compounds mentioned above quantitatively and rapidly. The procedure described in this paper is recommended after much investigation.

Description of Apparatus

Combustion tube and heating units—

The quartz tube (see Fig. 1) is approximately 100 cm long and 11 mm in diameter and is equipped with a side-arm at the rear end (mouth) for admission of carbon dioxide. The

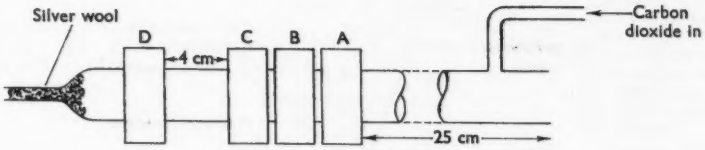

Fig. 1. Combustion tube and heating units

rear portion of the tube (25 cm) is empty and serves for the insertion of the sample in a platinum boat; it is heated by a movable furnace of the split type. The remaining portion of the tube is filled permanently and is heated by furnaces A, B, C and D, the first three being close to each other. The lengths of the furnaces and the sequences of the layers surrounded by them are: A, 16 cm long, filled with equal lengths of M.A.R., powdered cupric oxide, copper wire prepared by reducing M.A.R. cupric oxide wire with hydrogen, and cupric oxide powder; B, 28 cm long, filled with 18 cm of cupric oxide wire and 8 cm of copper wire; C, 10 cm long, filled with cupric oxide wire; D, 9 cm long, filled with 10- to 14-mesh granules of sodium fluoride puriss.[20] The gap between C and D, which is at least 4 cm (although there was no detrimental effect on the results when it was increased up to 15 cm), is filled with cupric oxide wire. The "beak" of the combustion tube is packed with M.A.R. silver wool. All layers are held in place by plugs of silica wool, the sodium fluoride layer being held rather loosely. The temperatures of furnaces A, B, C and D are adjusted to 750° (or between 750° and 800°), 850°, 600° and 180° C, respectively. When analysing compounds not containing fluorine and those easy to combust, furnace B can be used at 700° C. Furnace A is of the split type; B, C and D are tubular.

Oxygen generator—

A conical 75-ml suction flask (see Fig. 2) is placed in an inclined position between the bubbler (adjoining the carbon dioxide generator) and the combustion tube, and carbon dioxide passes over the surface of 50-volume hydrogen peroxide (M.A.R.). A piece of 60-mesh silver gauze suspended from a platinum hook at the end of a bent glass rod can be made to dip into or merely touch the surface of the hydrogen peroxide by turning the rod. Interruption

of the contact causes almost instant stoppage of the flow of oxygen. The supply of oxygen is regulated visually, *i.e.*, by observing the disappearance of the nitrogenous char in the combustion tube. Oxygen is produced intermittently, rather than continuously, to avoid excess of oxygen in the combustion train.

NITROMETER—

An ordinary semi-micro nitrometer has been used throughout. We found that the introduction of a few milligrams of black selenium powder into the 50 per cent. solution of potassium hydroxide prevents bubbles of gas from adhering to the surface of the mercury and breaks down foam at the potassium hydroxide meniscus; selenium partly dissolves in potassium hydroxide, forming a dark-reddish solution.

Also effective for this purpose is Pomatti's method, which involves agitation of a steel needle inside the nitrometer by means of a magnet.[21] The addition of mercuric oxide also prevents bubbles of gas from adhering to the surface of the mercury.

CARBON DIOXIDE GENERATOR—

The source of carbon dioxide is a Tucker generator,[22] which is connected to a bubbler containing a saturated solution of potassium carbonate to retain any drops of liquid carried over from the generator. The flow of gas is adjusted with a precision screw-clamp between

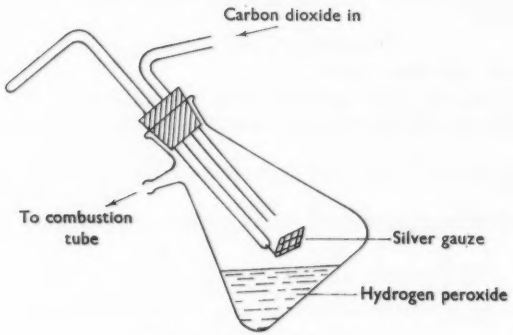

Fig. 2. Oxygen generator

the bubbler and the oxygen generator. The bubbler also serves as a useful detector of leakage; when the nitrometer is isolated by rotating the tail stopcock and the precision screw-clamp is fully opened, the appearance of bubbles would indicate leakage.

METHOD

PROCEDURE—

The mouth of the combustion tube is closed by a rubber stopper, and air is swept out with a fast stream of carbon dioxide, the gas escaping through the tail stopcock into the atmosphere, passing first through a small volume of water in a conical flask; a short piece of rubber tubing attached to the tail stopcock dips into the water. This simple arrangement acts as a hydraulic valve and prevents air from diffusing into the combustion tube. (After a determination, it is advisable to remove the conical flask and to close the stopcock, so as to prevent any water from being sucked into the tube as it cools.) The furnaces are switched on and allowed to attain the required temperatures. When small bubbles can be observed in the nitrometer, the platinum boat, containing between 10 and 20 mg of sample, is inserted in the combustion tube 5 to 10 cm from furnace A, with the stream of carbon dioxide still passing through the apparatus. The mouth of the combustion tube is closed, and carbon dioxide is passed until small bubbles again appear in the nitrometer. The flow of gas is then adjusted so that one or two bubbles rise in the nitrometer per second. The movable furnace is then pulled into position round the combustion tube.

In the presence of nitrogenous chars, the rate of combustion when the oxygen-injection technique is used is governed by the rate of disappearance of the char; as oxygen is generated,

the flow of carbon dioxide is appreciably slowed down (one or two bubbles per 2 seconds). When all the char has disappeared, generation of oxygen is stopped at once, and the slow stream of carbon dioxide is maintained for 3 to 5 minutes to ensure complete absorption of the excess of oxygen by metallic copper. At the same time, the movable heater is brought back to its starting position, and the empty part of the tube is re-traversed with it. After this, the tube is swept out with a fast stream (approximately 9 ml per minute) of carbon dioxide for 1 to 2 minutes, and the rate of flow is then decreased. When small bubbles appear once more, the movable heater is switched off and pushed clear of the combustion tube. The nitrometer is isolated, and, after 5 minutes, the volume of gas in it is read.

With a 10-mg sample of *p*-nitroaniline (calculated nitrogen content 20·28 per cent.) the entire operation took 15 minutes (nitrogen content found 20·1 and 20·2 per cent.). Some typical results are shown in Table I.

TABLE I

RESULTS AFTER COMBUSTION BY PROPOSED PROCEDURE

Unless otherwise stated under "Remarks," the temperatures of furnaces A, C and D were maintained at 750°, 600° and 180° C, respectively

Experiment No.	Sample	Temperature of furnace B, °C	Nitrogen content found, %	Nitrogen content calculated, %	Remarks
1	Trifluoroacetanilide (M.A.R.)	700	7·55	7·41	Slow combustion
2		850	7·54		
3		600	8·68		
4		850	7·52	7·41	Rapid combustion
5		700	7·93		
6		800	10·50	7·41	Furnace C at 800° C
7	Approximately (1 + 1) mixture of Teflon and *m*-dinitrobenzene (M.A.R.)	850	16·78	16·67	Oxygen injected; rapid combustion
8			15·26		No oxygen injected; slow combustion
9			16·76		No oxygen injected; boat placed in close contact with permanent CuO filling
10	*p*-Nitroaniline (M.A.S.)	700	20·29	20·28	Oxygen injected; rapid combustion
11	Sulphanilic acid (A.R.)		8·11	8·09	
12	8-Hydroxyquinoline (M.A.S.)		9·59	9·65	Oxygen injected; very slow combustion

BLANK TEST—

A check was carried out under rather artificial conditions by passing a very rapid stream of carbon dioxide through the combustion train for 30 minutes and keeping the temperature of furnace B at 850° C. A blank of less than 0·02 ml was obtained in the nitrometer. With the normal Dumas temperature setting (furnace B at 700° C), the blank was hardly perceptible, even with a magnifying lens, *i.e.*, it was practically zero.

DISCUSSION OF THE METHOD

According to Clark and Rees,[5,23] the ordinary Dumas micro-determination of nitrogen can be used without modification for fluorine-containing compounds. In our view, an increase in the temperature of combustion over that ordinarily used is imperative. Obviously, the temperature requirements will vary somewhat with the nature of the compound, *e.g.*, whether it is partly or completely fluorinated[7]; for example, we found that heptafluorobutyramide was more resistant to pyrolytic attack than was trifluoroacetanilide. As a general rule, we established that the lower limit of temperature for complete combustion was about 800° C; the upper limit was 900° C or less, being determined by the extent to which the copper oxide attacked the silica tube.[14] Not infrequently, this produced cracks in the tube, and we therefore recommend that the temperature of furnace B be maintained at 850° C.

There are three combustion zones in our apparatus. First, the pre-combustion zone (heated by furnace A), containing powdered copper oxide to assist combustion. Here, most substances burn quantitatively when combusted slowly, but fluorine-containing compounds

tend to behave differently (see experiment No. 3, in Table I). The temperature setting of A is not altered throughout. Second, the "finishing" zone (heated by furnace B), the function of which is two-fold; it completes the combustion of "awkward" compounds (with B at the high-temperature setting) and helps to carry out combustions rapidly for all compounds (with B at the high- or middle-temperature setting). Third, the "eliminating" zone (heated by furnace C), which helps to restore the carbon dioxide balance prevailing in the normal Dumas method,[16,17] so eliminating carbon monoxide[24 to 29] produced in the hot "finishing" zone.[30] Experiment 6 (see Table I) emphasises the well known fact that under high-temperature conditions the Dumas method is not very satisfactory.[5]

As furnaces A and B differ relatively little from each other functionally they could conceivably be amalgamated into one large high-temperature heater. We found it more practicable to have them separate, especially as A is of the split type and hence easy to inspect visually.

The introduction of oxygen into the Dumas train has, in our experience, no effect on C–F cleavage.[31] The presence of hydrogen, however, according to Milton and Waters,[32] seems to be necessary for the satisfactory analysis of fluorine-containing compounds. Presumably, fluorine formed by initial C–F cleavage combines with hydrogen to form hydrogen fluoride, which, with silica, gives silicon tetrafluoride and water vapour. Only traces of moisture, always present in the Dumas system, would therefore be required to initiate this chain reaction.

This raises the problem of the disposal of silicon tetrafluoride. If it reaches the nitrometer undecomposed, it will be quantitatively absorbed by the potassium hydroxide solution, but we found that it might be partly hydrolysed by traces of moisture, resulting in deposition of silica near or in the stopcock and so blocking the flow of gas. This is effectively prevented by a layer of sodium fluoride[33] in the "beak" end of the combustion tube (maintained at 180° C by furnace D). After having carried out determinations of nitrogen both with and without this sodium fluoride, we strongly recommend its use in the routine analysis of fluorine-containing compounds.

For substances difficult to combust, giving nitrogenous chars, the addition of controlled amounts of oxygen to the cupric oxide - copper-filled tube is, in our experience, satisfactory. Our oxygen generator differs from that proposed by Unterzaucher[12] in that carbon dioxide does not bubble through a solution of hydrogen peroxide; the flow of oxygen is therefore independent of the flow of carbon dioxide, and oxygen is produced intermittently instead of continuously. Further, we use silver instead of platinum, the 60-mesh silver gauze being relatively cheap and readily available. The catalytic activity of silver is similar to that of platinum; the rate of decomposition of hydrogen peroxide on silver is 10^7 times that on an inert material such as polythene.[34]

Experiment No. 9 in Table I shows a rather interesting observation: when no external oxygen is available, errors due to the formation of nitrogenous chars can often be decreased by placing the platinum boat containing the sample close to the cupric oxide powder of the permanent filling. This technique, although not a complete remedy, is significantly simple.

The achieved increase in the rate of combustion we attribute mainly to increases in length of tube and temperature, both of which favour more thorough combustion; the greatest danger in any rapid method is the occurrence of incompletely burnt gaseous products of decomposition in the nitrometer.[17] The active life of the combustion tube in our apparatus is about sixty determinations, with an average weight of sample of 15 mg.

We thank Professor R. N. Haszeldine for the communication referred to on p. 512 and Mr. B. Woodbridge for carrying out various timing operations.

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Determination of Added Borates in Mixed Fertilisers

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A method for determining borates in fertilisers in the range 0·1 to 1 per cent. of boron is described. Phosphate is removed by adding bismuth nitrate solution, and the "identical-pH" method is used to detect the end-point of the subsequent mannitol - boron titration.

Interest in the manufacture of "compound" fertilisers to which borates have been added is increasing, and this has focused attention on the analytical problem of accurately determining the borate present. The amount added is more than a "trace" and may generally be within the range 0·1 to 1 per cent. of boron. Current methods of determining borates are either photometric or volumetric. Most of the photometric methods are perhaps more suitable for "traces" than for larger amounts, and it is thought that a volumetric method will be preferable. These methods depend on the formation and titration of glyceroboric acid or, better, mannitoboric acid, and this necessitates prior separation of the borate from interfering substances, including phosphate. Distillation as methyl borate and hydrolysis of the distillate is well known, but it is time-consuming and difficult to carry out quantitatively. The American Association of Agricultural Chemists[1] recommend two methods, one for acid-soluble and the other for water-soluble boron. In the first, phosphate is removed with lead nitrate, the excess of lead (and also calcium) being removed by sodium hydrogen carbonate. Finally, the boric acid is titrated by adjustment of the pH to 6·3, addition of mannitol and titration to the same pH value with sodium hydroxide solution ("identical-pH" method). The water-soluble boron is extracted by hot water, phosphate is removed with barium hydroxide solution, and ammonia is removed by boiling for at least 1 hour. After filtration and removal of carbon dioxide, the solution is made neutral to methyl red, mannitol is added, and the mannitoboric acid titrated to the phenolphthalein end-point.

These methods can produce precipitates that are difficult to handle (even if "filter aids" are used), and in our hands the results were not as reproducible as one could wish. We therefore considered the possibility of using some other reagent to remove phosphate from the solution. Among the known precipitants for phosphate, bismuth nitrate in dilute acid solution seemed promising. As long ago as 1860, Chancel[2] determined phosphate by precipitation as bismuth phosphate and noticed that the crystalline precipitate settled and filtered well. Rathje[3] had proposed to titrate phosphate with bismuth nitrate solution, locating the end-point by the orange colour of bismuth iodide, but pointed out that chlorides and sulphates interfere through formation of basic salts. It occurred to us that the use of bismuth would

TABLE I

BORATE FOUND IN COMMERCIAL FERTILISER

Sample No.	Borate content found, as boron, by— analyst A, %	analyst B, %	analyst C, %
1	0·171	0·173	0·177
	0·169	0·174	
	0·172	0·176	
	0·172	0·177	
2	0·160	0·157	0·159
	0·159	0·158	
	0·160	0·161	
	0·161	0·162	

also have the advantage that the excess could be readily removed as basic salts by increasing the pH and diluting the solution. A few experiments showed that a separation was possible, but also showed that an excess of bismuth was necessary over that equivalent to the phosphate present, presumably because of the formation of basic salts.

EXPERIMENTAL

Each of a series of 2·5-g portions of a commercial "compound" fertiliser containing 10 per cent. of P_2O_5 (based on superphosphate) was dissolved in diluted nitric acid, and the resulting solutions were treated with different volumes of a 22 per cent. w/v solution of bismuth nitrate (see "Method"). The amount of phosphate remaining in solution was then determined spectrophotometrically; the results were—

Volume of bismuth nitrate solution used, ml. .	50	45	40	35	30	25	20
Proportion of original P_2O_5 left in solution, %	0·2	2·0	3·5	9·5	25·0	54·0	76·5

These results show that only with a volume of the bismuth nitrate solution equivalent to 5 ml for each 1 per cent. of P_2O_5 in the fertiliser is removal of phosphate virtually complete.

To obtain a crystalline precipitate that settles well, the bismuth nitrate solution must be added slowly to the hot acid solution of the fertiliser, with frequent agitation; after the addition is complete, the pH of the solution is about 1·7. It was found most convenient to allow the solution to cool, dilute it to a given volume and then filter an aliquot part rather than to wash the bismuth phosphate. That this does not lead to loss of borate is shown by the results on p. 519. Excess of bismuth in the aliquot is removed by addition of a small excess of sodium hydroxide. This precipitate (basic salts of bismuth) is washed, and the borate is then titrated in the filtrate by the "identical-pH" method after removal of carbon dioxide. For a fertiliser containing 0·15 to 0·2 per cent. of boron, the titre of 0·02 N sodium hydroxide is in the range 7 to 10 ml. There is a slight blank value; if possible, this should be determined by analysing a similar fertiliser to which no borate has been added. If this is not possible, an average blank value of 0·15 ml of 0·02 N sodium hydroxide can be used.

We have only concerned ourselves with total or acid-soluble borate. It is doubtful what significance would attach to water-soluble borate, and one or two preliminary experiments suggested that the method of extraction influenced the results. Although the "identical-pH" method has often been treated as empirical, our results justify the use of the stoicheiometric factor under our conditions.

REPRODUCIBILITY OF RESULTS

To two samples of fertiliser (A based on superphosphate and B based on ammonium

phosphate) known amounts of sodium tetraborate were added, and the boron contents were determined as described under "Procedure"; the results were—

Sample	..	..	A			B		
Boron added, %	..	..	0·517	0·319	0·165	0·958	0·451	0·229
Boron found, %	..	..	0·517	0·315	0·162	0·962	0·450	0·231

Two samples of a commercial borated fertiliser (based on superphosphate) were analysed by three analysts, the third of whom made only one determination on each sample and had no previous knowledge of the method; the results are shown in Table I. These results, together with those above, show satisfactory reproducibility.

The time required for a determination in duplicate is about 3 hours.

Method

Reagent—

Bismuth nitrate solution—Dissolve 22 g of $Bi(NO_3)_3.5H_2O$ in 8 ml of concentrated nitric acid, with slight warming if necessary, and dilute to 100 ml with water.

Procedure—

Weigh out 2·5 g of sample, transfer to a 400-ml beaker, add 2 ml of nitric acid and 50 ml of water, stir, warm, and dilute to 100 ml with water. Warm the solution to 80° or 90° C (do not boil, as boric acid is volatile in steam), and slowly add from a burette, with continuous stirring, 5 ml of the bismuth nitrate solution for each 1 per cent. of P_2O_5 present in the fertiliser. Keep the solution hot during precipitation. Allow the precipitate to settle, cool, wash into a 250-ml calibrated flask, and dilute to the mark. Filter through a dry Whatman No. 40 filter-paper, rejecting the first few millilitres, and, by pipette, place 100 ml of the filtrate in a beaker. Add a few drops of bromothymol blue indicator solution and then a 10 per cent. solution of sodium hydroxide, with thorough stirring, until the indicator turns blue. Separate the precipitate on a Whatman No. 541 filter-paper, carefully wash it several times with cold water, and combine the washings with the filtrate (the total volume should be about 150 to 200 ml). Adjust the pH to about 5 by adding 5 per cent. nitric acid, heat to about 90° C (do not boil), and stir vigorously to remove carbon dioxide.

Cool, place in the solution the electrodes of a suitable pH meter, and adjust the pH to 6·3; first use 10 per cent. sodium hydroxide solution, and finally bring the pH exactly to 6·3 with carbon dioxide-free 0·02 N sodium hydroxide. Add 10 g of mannitol, and again bring the pH to 6·3 with the 0·02 N sodium hydroxide. Continue to add 10-g portions of mannitol and to re-adjust the pH to 6·3 until, after the final addition of mannitol, the pH remains constant at 6·3. (For samples containing up to 0·5 per cent. of boron, 20 g of mannitol are usually sufficient.) The total amount of 0·02 N sodium hydroxide used after the additions of mannitol corresponds to the amount of boron present in the solution.

Carry out a blank determination on a similar type of fertiliser to which borate has not been added, and subtract the blank titre from the titre previously obtained; if a "blank" sample is not available, deduct 0·15 ml from the titre of 0·02 N sodium hydroxide. Calculate the borate content, as boron, of the sample from the equation—

$$\text{Boron content, \%} = (A - B) \times 0{\cdot}000216 \times 100$$

in which A and B are the titres of 0·02 N sodium hydroxide in the sample and blank determinations, respectively.

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The Determination of Dihydroxybenzenes by Liquid – Liquid Partition Chromatography

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Liquid - liquid partition chromatography is slow, largely because of the time involved in identifying and determining components in the eluate. With the apparatus described, most of these time-consuming operations are avoided by continuously recording the percentage of ultra-violet light of fixed wavelength transmitted by the eluate. By this means, the emergence from the column of separated components is indicated by the appearance of peaks on the recorder chart. Some components can be identified and determined directly from the trace by the relative positions of and areas under the peaks. For others, an automatic fraction collector is used, and the trace serves as a guide for blending fractions for subsequent ultra-violet analysis. The method has been applied mainly, but not exclusively, to the determination of dihydric phenols in aqueous and organic solutions.

THE phenolic fraction extracted from coal-tar distillates boiling over the range 230° to 270° C is essentially a complex mixture of monohydroxy aromatics containing minor amounts of dihydroxybenzenes. As the latter may have some bearing on the properties and utilisation of such a fraction, a method was required for determining individual dihydroxybenzenes in the presence of much larger amounts of monohydric phenols. Such a method would also be of value in classifying aqueous phenolic effluents and in evaluating procedures for treating them; for this purpose, the method should be applicable to a dilute aqueous solution of the sample. Chromatographic procedures were clearly suggested, and liquid - liquid partition was preferred to paper chromatography because it could more easily be made quantitative. Such a method had already been successfully devised[1] and appeared to be suitable for our purpose. The chief disadvantages of the column procedure were the slowness of operation and the time required for identifying and determining the eluted components. In view of this, a semi-automatic apparatus was designed to work without attention overnight and to record a trace showing the eluted components. The amounts of individual dihydric phenols could then be determined after blending fractions (the trace being used as a guide) or sometimes directly from the trace.

Although the apparatus has been constructed for determining dihydric phenols, it can also be used for other chromatographic determinations, with a suitable choice of phases, provided that a wavelength can be found in the visible or ultra-violet region at which the solvent transmits light appreciably more than do the components to be measured.

Description of apparatus

Solvent handling—

The device for supplying the eluting agent is shown in Fig. 1. The eluting agent (cyclohexane) is supplied from a 1-litre flask, A, equipped with a bottom off-take leading to a tap and a male spherical joint (14 mm diameter). When gradient elution is used, the more polar solution (20 per cent. v/v of n-butyl alcohol in cyclohexane) is contained in a second 1-litre flask, B, and is continuously supplied to flask A via siphon tube C (2 mm i.d.). A stirrer in flask A ensures mixing, and a side-tube is provided to prime the siphon by slight air pressure. It has been found that the siphon gives a more linear change in concentration with volume of eluting solution than does the more conventional arrangement of a separating funnel mounted on the flask so that the flask remains full.

When analysing phenolic mixtures it is often desirable to use only pure cyclohexane as eluting agent during the early stages of elution. A delay before the siphon begins to operate can be achieved by fitting a non-return valve of the type shown at D in Fig. 1. A B10 air leak is cut off close to the joint, and a 3/16-inch ball-bearing is ground in with emery powder. Final grinding is done with very fine emery, a second identical ball being used,

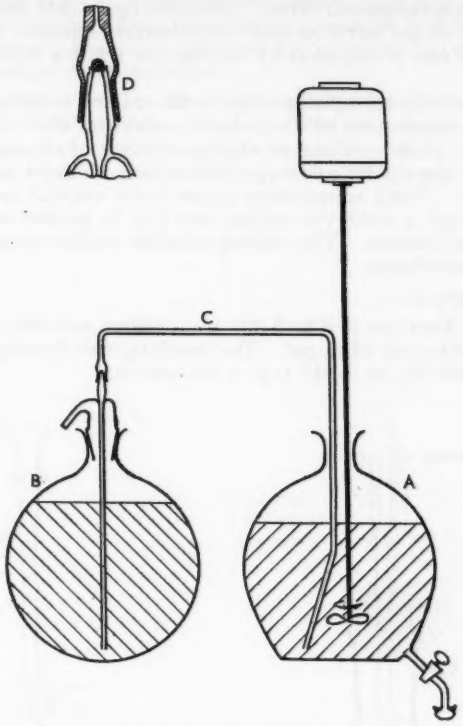

Fig. 1. Details of supply of eluting solution

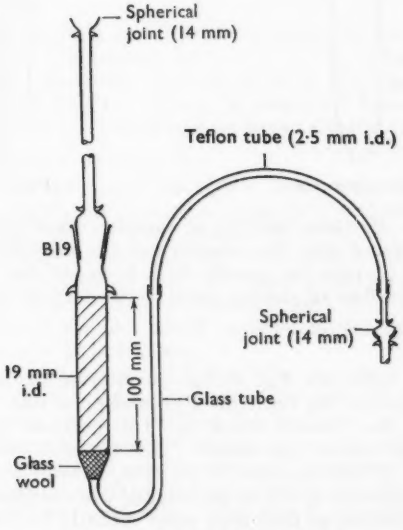

Fig. 2. Details of pre-column

and this ball is used in the completed valve. The valve opens only under a positive hydrostatic head of about 40 mm of the butyl alcohol - cyclohexane solution, so that the delay before the gradient is applied can be adjusted by varying the relative heights of the flasks and the liquids in them.

It is important that eluting agent passing to the column is saturated with the stationary phase (water) at the temperature of the column, otherwise each component may give rise to two partly resolved peaks. When an eluting solution of changing composition is used, water is added to the more polar solution to the extent of about two-thirds of the amount required for saturation. Final adjustments to the water content are then made by passing the eluting agent through a small pre-column (see Fig. 2) packed with the same stationary phase as the analytical column. The eluting solution attains equilibrium by accepting or losing water in this pre-column.

INTRODUCTION OF SAMPLES—

Aqueous samples—Portions (0·5 to 5 ml) are acidified and then mixed with twice their weight of dry 60- to 80-mesh silica gel. The resulting free-flowing powder is put into an adapter (see Fig. 3) that fits on to the top of the column.

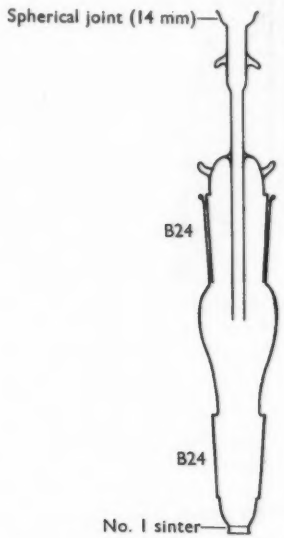

Fig. 3. Adapter for aqueous samples

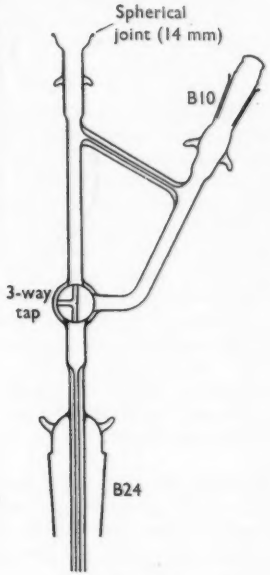

Fig. 4. Sample injector

Non-aqueous samples—Portions (0·5 ml) of samples dissolved in a cyclohexane - butyl alcohol mixture are introduced into the side-limb of the injection device shown in Fig. 4. A three-way tap is set to by-pass the sample limb until the flow has been adjusted. The tap is then turned, and the flow of eluting agent is diverted to wash the sample on to the column.

PREPARATION OF COLUMN—

The chromatographic tube (see Fig. 5) has an internal diameter of about 14 mm and is 640 mm long. It is fitted at the top with a ground-glass B24 socket and at the bottom with a B10 cone having a No. 1 sinter below it. This position for the sinter decreases the space in which fractions of eluate can diffuse. For the analysis of dihydric phenols, the tube is packed to a depth of 570 mm with 55 per cent. w/w of water on acid-washed 60- to 80-mesh silica gel. This system is based on the original work of Blackburn, Barker, Catchpole and Hollingworth[1] and is similar to that used more recently by Barker and Hollingworth[2,3] concurrent with the developments described here.

The most satisfactory method of packing the column is to add the correct proportion of water to a stirred slurry of dry silica gel in a large volume of cyclohexane and to continue the stirring for a short time. The slurry obtained can be packed to form a column showing no "bands" by the technique described below.

The chromatographic tube is filled with cyclohexane and connected via a siphon tube (see Fig. 6) to a 250-ml flask containing about 60 g of the prepared gel in about 150 ml of cyclohexane. A micro pump (obtainable from the Distillers Company Ltd.) is then used to pump cyclohexane into the flask at 1500 ml per hour so as to force the

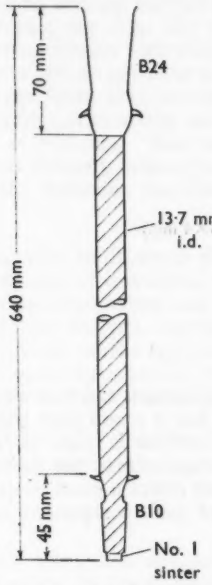

Fig. 5. Details of column

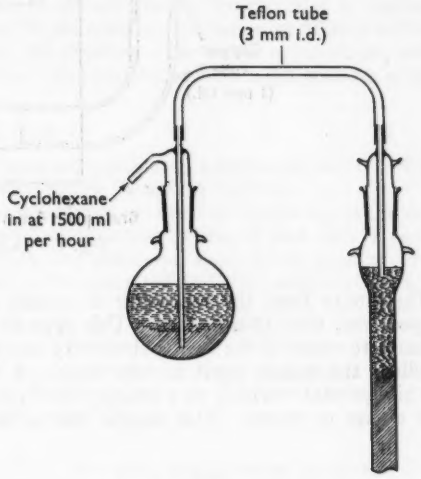

Fig. 6. Column-packing device

slurry through the siphon tube and into the column; the surplus cyclohexane running from the bottom of the column can be re-cycled. It is sometimes necessary to tap the 250-ml flask lightly to ensure an even flow of slurry. When packing has been completed, the top surface of the column is protected from disturbance by a disc of filter-paper, and the level of cyclohexane is never allowed to fall below this disc.

COLLECTION AND MEASUREMENT OF ELUATE—

The eluate from the column passes through a Teflon tube (1·5 mm i.d.) to a cell constructed as shown in Fig. 7 from silica plates secured with Araldite adhesive to a brass plate 4 mm thick. This assembly fits in the cell-well of a Unicam SP500 spectrophotometer modified for automatic scanning as described by Shrewsbury.[4] In this work, the instrument is set at a fixed wavelength (2800 A), and the percentage transmission is recorded on a chart moving at 1 inch per hour. (The cyclohexane used must have a low optical density at all wavelengths accessible to the instrument; commercial grades must generally be purified before use by passage through dry silica gel.)

From the cell, the eluate passes into a Teflon tube (1·5 mm i.d.) coupled by a B10 joint to a flow controller (see Fig. 8). Accurate control is obtained by adjusting the height of a stainless-steel wire (17 s.w.g.) and so varying the restriction to flow imposed by it. It is an advantage to file the wire so that its cross-sectional area is decreased towards its lower tip.

The rate of flow is measured by a simple calibrated flowmeter of the type shown in Fig. 9, which indicates the hydrostatic head of eluting solution required for a corresponding rate of flow through a fixed capillary restriction. The flow is normally in the range 50 to 80 ml per hour.

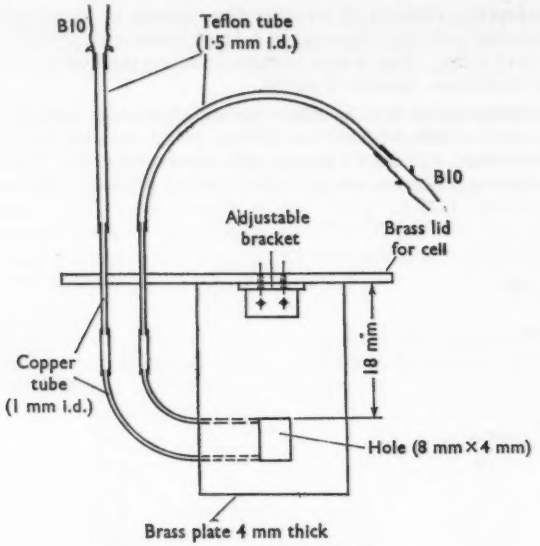

Fig. 7. Details of cell

The eluate from the flowmeter is usually collected with a Shandon fraction collector equipped with fifty 15-ml tubes. This type of fraction collector has a scoop that will take the eluate to waste if the mains electricity supply is interrupted, and use is made of this by controlling the mains input in two ways. A time switch stops and starts the collection, and a Simmerstat working on a time cycle of just over 1 minute will direct a fixed proportion of the eluate to waste. This means that a known proportion of each component can be

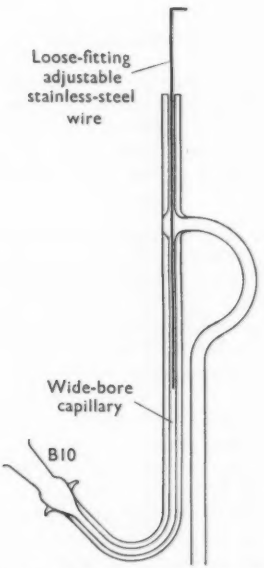

Fig. 8. Details of flow controller

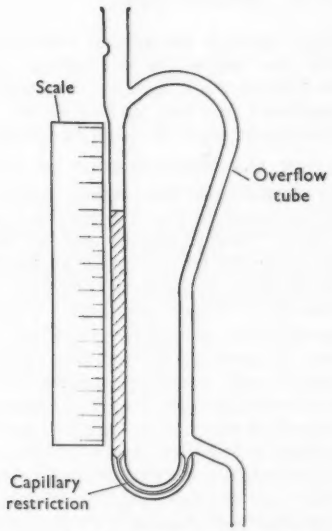

Fig. 9. Details of flowmeter

collected during a long run without exceeding the volumetric capacity of the fraction collector. Used in conjunction, the two controls permit complete collection of part of the eluate and partial collection of the remainder. Each change of fraction is marked on the trace by a small "spike." This is achieved by connecting a micro switch to a pawl that keeps contact with the notched rim of the turn-table. During each change of fraction, the signal to the recorder can then be partly shorted by a 9000-ohm resistor.

Operation

As the spectrophotometer is used for other purposes during the day, it has been convenient to operate the column overnight as described below.

During the day, the pre-column is re-packed, and butyl alcohol is washed from the analytical column with about 500 ml of purified cyclohexane; the rate of flow may be as high as 200 to 300 ml per hour and no air pressure is required. The flow is then adjusted to about 70 ml per hour, and, during the last half-hour of the afternoon, the recorder is started, set to zero for dark current and then set at about 90 per cent. transmission at 2800 A with a slit width of 0·45 mm. The sample is introduced, the fraction collector is switched on, and the gradient siphon is primed (both flasks being full). The apparatus is then left without attention until the following morning.

Results

In order to illustrate the procedure, the trace for a prepared mixture is shown in Fig. 10, the amounts of individual phenols present being in the range 0·2 to 2 mg.

It has been found that the butyl alcohol is not eluted evenly, but builds up on the column and, during the run, suddenly "breaks through" at a concentration of just over 1 per cent. At this point on the trace a spurious peak occurs, the nature of which is not known. It may be produced by traces of impurities adsorbed on the silica gel and then displaced as a sharp band by the butyl alcohol.

Quantitative analysis—

The amount of a component present in a peak is given by the expression—

$$\text{Weight present, mg} = \frac{kAtq}{El}$$

in which k is a constant, A is the maximum height of the peak expressed as an optical density, t is the time in hours for the elution of the peak (the intercept along the base-line between the tangents to the sides of the peak), q is the rate of flow in millilitres per hour, E is the extinction coefficient ($E^{1\%}_{1\,cm}$) at the wavelength used and l is the thickness of the cell in centimetres.

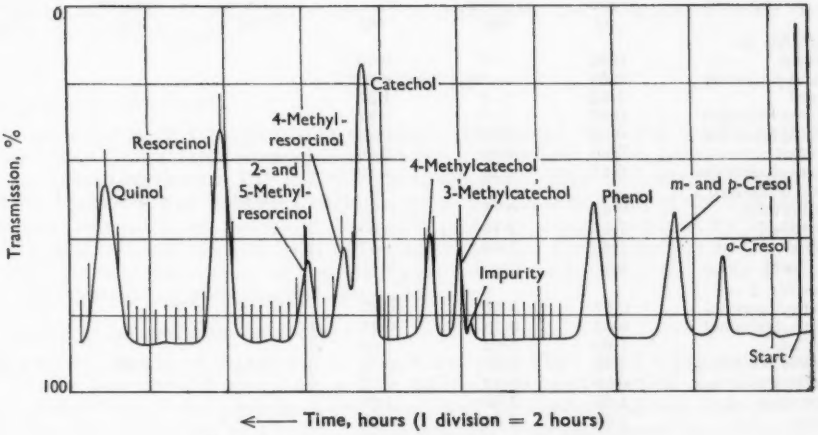

Fig. 10. Trace recorded for a prepared mixture

By calibration with prepared mixtures, the value of k was found to be about 5·7. For a true Gaussian peak,[5] k should be equal to 6·27; the lower value is a purely empirical factor, which takes account of the flow conditions in the cell. In practice, it is convenient to tabulate values of k/El for the series of compounds to be determined, and the values used at 2800 A for some common phenols are shown in Table I.

TABLE I

VALUES OF k/El FOR VARIOUS PHENOLS

Phenol	Value of k/El, mg per ml	Phenol	Value of k/El, mg per ml
Phenol	0·142	Catechol	0·070
o-Cresol	0·108	3-Methylcatechol	0·113
m-Cresol	0·088	4-Methylcatechol	0·077
p-Cresol	0·075	3,4-Dimethylcatechol	0·060
2,3-Xylenol	0·114	3,6-Dimethylcatechol	0·112
2,4-Xylenol	0·087	4,5-Dimethylcatechol	0·097
2,5-Xylenol	0·111	3-Ethylcatechol	0·118
2,6-Xylenol	0·163	4-Ethylcatechol	0·085
3,4-Xylenol	0·098	Resorcinol	0·089
3,5-Xylenol	0·135	2-Methylresorcinol	0·186
2-Ethylphenol	0·105	4-Methylresorcinol	0·072
3-Ethylphenol	0·097	5-Methylresorcinol	0·127
4-Ethylphenol	0·103	2,4-Dimethylresorcinol	0·257
		4-Ethylresorcinol	0·079
		Quinol	0·095

As an alternative quantitative method, ultra-violet analysis of collected fractions is slower, but slightly more accurate; it is essential when the identity of a peak is in doubt.

In applying methods of quantitative analysis (especially by measurement of areas) it is important to allow for the polarity of the solvent used. The presence of butyl alcohol is likely to produce a considerable change in the spectrum of a phenol in cyclohexane and

TABLE II

RESULTS FOUND FOR PREPARED MIXTURES

Mixture No. 1 is that for which the trace is shown in Fig. 10. Average values for extinction coefficient were used in the two instances when the components were not resolved

Component	Amount of component present, mg	Amount of component found by— ultra-violet analysis, mg	Amount of component found by— measurement of area, mg	Total amount of collected dihydroxybenzenes— present, mg	Total amount of collected dihydroxybenzenes— found by ultra-violet analysis, mg	Total amount of collected dihydroxybenzenes— found by measurement of area, mg
Mixture No. 1—						
o-Cresol	0·34	—*	0·33			
m- and *p*-Cresols	0·65	—*	0·64			
Phenol	1·52	—*	1·26			
3-Methylcatechol	0·30	—*	0·31			
4-Methylcatechol	0·32	0·34	0·33			
Catechol	1·86	2·00	2·06			
4-Methylresorcinol	0·31	0·28	0·39			
2- and 5-Methylresorcinols	0·62	0·71	0·67	6·46	6·73	6·87
Resorcinol	1·67	1·76	1·85			
Quinol	1·68	1·64	1·57			
Total	9·27	—	9·41			
Mixture No. 2—						
3-Methylcatechol	0·57	0·61	0·57			
4-Methylcatechol	0·15	0·19	0·16			
Catechol	1·60	1·60	1·68			
4-Methylresorcinol	0·46	0·49	0·50	6·51	6·63	6·57
5-Methylresorcinol	0·26	0·42	0·27			
Resorcinol	1·79	1·78	1·80			
Quinol	1·68	1·54	1·59			

* Fraction not collected.

will therefore affect the extinction coefficient at the wavelength used during the run. The extinction coefficient of the component in the correct solvent must always be used.

Quantitative results for two prepared mixtures are shown in Table II to indicate the accuracy to be expected from the method.

I thank the Directors of the Midland Tar Distillers Ltd. for permission to publish this paper and Mr. D. D. Shrewsbury for designing and constructing the cell and carrying out the necessary ultra-violet analyses.

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Thin-layer Chromatography of 3,5-Dinitrobenzoates

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The behaviour of some 3,5-dinitrobenzoates on chromatoplates has been studied; the R_F values are compared with the R_B values found by measuring the locations of the compounds against that of a standard substance. A procedure is described for preparing the dinitrobenzoates of hydroxyl compounds present in very dilute solutions.

The method is suitable for detecting small amounts of these compounds in water and in the presence of large amounts of methanol or ethanol. Small amounts of the dinitrobenzoates can be purified, since the esters can easily be separated from the excess of unchanged reagent.

In a study of steam-volatile alcohols and phenols in foods, a simple and rapid method was required for separating and purifying certain 3,5-dinitrobenzoates prepared by reaction of the hydroxyl compounds with 3,5-dinitrobenzoyl chloride. Results obtained in the separation of 2,4-dinitrophenylhydrazones[1] prompted us to investigate the behaviour of some dinitrobenzoates on chromatoplates.

Experimental

Apparatus and technique—

Chromatoplates were prepared, as described previously,[1] by using Stahl's apparatus,[2] with silica gel G (obtainable from E. Merck A.G.) as adsorbent. The plates were activated before use by heating them at 110° to 120° C for 15 minutes. After the plates had been allowed to cool on a large plate of glass for 5 minutes, spots (usually 5 to 10 μl) of the solutions being investigated were placed on them, and the treated plates were put into a tank for development within 10 minutes after removal from the heated cabinet. The atmosphere in the tank was kept saturated with the vapour of the developing solvent by covering the walls of the tank with strips of filter-paper dipping into the solvent.

Formation of 3,5-dinitrobenzoates—

A weighed amount of the alcohol or phenol was placed in a small round-bottomed flask, slightly more than the calculated amount of 3,5-dinitrobenzoyl chloride and then 10 ml of benzene and 0·1 ml of dry pyridine were added, and the contents of the flask were heated under reflux for 30 minutes to 1 hour. When cool, the reaction mixture was extracted with 25 ml of 0·1 N sulphuric acid, then with 25 ml of a 0·5 to 1 per cent. solution of sodium

carbonate and finally with water. The aqueous layers were discarded, and the benzene solution was evaporated to dryness under reduced pressure in a bath of boiling water. The residue was covered with a few millilitres of benzene, so that a saturated solution of the ester was obtained.

Solutions of the esters in methanol were not stable; after some time, these produced a second spot on the chromatogram, in the position of that formed by the 3,5-dinitrobenzoate of methanol. The unchanged dinitrobenzoyl chloride is converted into dinitrobenzoic acid. However, it is not necessary to remove this acid by extraction with sodium carbonate solution, as it remains at the start in the solvents used for developing the chromatograms.

SOLVENT SYSTEMS—

The solvent systems tested included a (1 + 1) mixture of benzene and light petroleum (boiling range 60° to 80° C), hexane containing 15 or 25 per cent. of ethyl acetate and toluene containing 10 per cent. of ethyl acetate. The results for the 3,5-dinitrobenzoates of normal

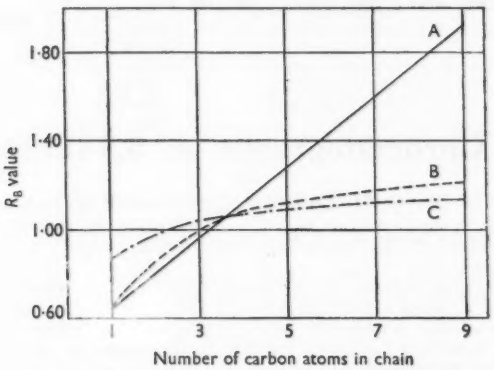

Fig. 1. R_B values for 3,5-dinitrobenzoates of aliphatic alcohols as a function of chain length: curve A, benzene - light petroleum mixture (1 + 1) as solvent; curve B, toluene containing 10 per cent. of ethyl acetate as solvent; curve C, hexane containing 15 per cent. of ethyl acetate as solvent

aliphatic alcohols when these systems were used are summarised in Fig. 1. The system giving the best results was the benzene - light petroleum mixture, and this was subsequently used throughout.

COMPARISON BETWEEN R_F AND R_B VALUES—

During our work on the thin-layer chromatography of 2,4-dinitrophenylhydrazones, we found that R_F values were dependent on the distance travelled by the solvent front, and we defined[1] the R_B value by the expression—

$$R_B = \frac{\text{Movement of substance from start, mm}}{\text{Movement of butter yellow from start, mm}}.$$

(Nicolaus[3] also recently recommended the use of a standard substance for measuring the positicns of the spots.)

The R_B and R_F values for the dinitrobenzoates of methanol and hexanol were measured under different conditions; two adsorbents were used, Merck's silica gel G and silica gel obtained from Mallinckrodt. The latter adsorbent was prepared by mixing 25 g of Mallinckrodt's silica gel with 5 g of gypsum in a mortar and then adding 60 ml of distilled water. From this slurry, chromatoplates were prepared in the way described for silica gel G.[1]

Table I shows the results of a series of experiments in which the R_B and R_F values for the 3,5-dinitrobenzoate of hexanol were compared. The largest difference between R_F values was 30·1 per cent. and that between R_B values was 18·6 per cent. The results for the 3,5-dinitrobenzoate of methanol were essentially the same, the largest differences between R_F and R_B values being 50·0 and 14·3 per cent., respectively. For this reason, we use R_B values

in preference to R_F values for defining the positions of the compounds on the chromatograms.

The R_B values of the aliphatic alcohols can be seen from Fig. 1, and those for some other hydroxyl compounds are listed in Table II.

TABLE I

R_B AND R_F VALUES FOR THE 3,5-DINITROBENZOATE OF HEXANOL

Silica gel used	Time of activation, minutes	Distance of solvent front from start, cm	Number of experiments	Mean R_F value	Standard deviation of R_F value	Mean R_B value	Standard deviation of R_B value
Merck	15	10	9	0·39	0·006	1·34	0·020
Mallinckrodt ..	15	10	6	0·405	0·012	1·28	0·023
Merck	30	9·5	6	0·26	0·011	1·27	0·016
Merck	5	14	6	0·284	0·002	1·19	0·014
Mallinckrodt ..	5	9·5	10	0·494	0·018	1·24	0·021
Merck	60	6	6	0·273	0·020	1·50	0·023

TABLE II

R_B VALUES FOR VARIOUS ALCOHOLS AND PHENOLS

Compound, as 3,5-dinitrobenzoate	R_B value	Compound, as 3,5-dinitrobenzoate	R_B value
Phenol	0·76	Eugenol	0·55
Thymol	1·42	Isoeugenol	0·56
1-Naphthol	0·89	Citronellol	1·69
2-Naphthol	0·93	Geraniol	1·26
o-Cresol	1·00	Terpineol	1·41
m-Cresol	1·02	Furfuryl alcohol	0·71
p-Cresol	1·05	Benzyl alcohol	0·76
		Maltol	0·92

METHOD

PREPARATION OF 3,5-DINITROBENZOATES FROM DILUTE SOLUTIONS—

As the hydroxyl compounds were always obtained as dilute solutions in water or ethanol, it was difficult to prepare the derivatives directly. Although some success was achieved by using Holley and Holley's method,[4] in extremely dilute aqueous solutions this method was not very sensitive. It was especially difficult to prepare the dinitrobenzoates of phenolic compounds.

A large excess of ethanol is often present in steam-distillates from foods; in such distillates the minor components are completely masked. However, results were satisfactory when these distillates were extracted two or three times with small amounts of pentane, methanol and ethanol then remaining almost completely in the aqueous layer; even small amounts of higher alcohols and phenols were easily extracted.

The pentane extract was dried over anhydrous sodium sulphate for some hours, and then decanted into an Erlenmeyer flask containing 10 to 20 ml of benzene, 0·5 g of 3,5-dinitrobenzoyl chloride and 1 to 2 ml of dry pyridine. The mixture was heated under reflux for 30 minutes to 1 hour and, when cool, was extracted successively with 25-ml portions of 0·1 N sulphuric acid, a 5 per cent. solution of sodium carbonate and water. The organic layer was then evaporated to dryness under slightly reduced pressure in a bath of hot water. To the dry residue were added 2 to 4 ml of benzene, 5 to 10 μl of this saturated solution were placed on an activated plate, and the dinitrobenzoates were separated by development in (1 + 1) benzene - light petroleum mixture. The developed chromatogram was sprayed with a 1 per cent. solution of 1-naphthylamine in ethanol; the benzoates then became visible as yellow to orange spots.

DISCUSSION OF THE METHOD

Fig. 2 shows two chromatograms obtained with mixtures of known composition; 3,5-dinitrobenzoic acid, which may be present in relatively large amounts, does not affect the separation, as it remains at the start when the benzene - light petroleum solvent is used.

Since, for separation, R_B values must differ by at least 0·3 to 0·4, the method is clearly unsuitable for separating the members of homologous series of alcohols or phenols. However, very small amounts of higher alcohols in ethanol can easily be detected. Small amounts

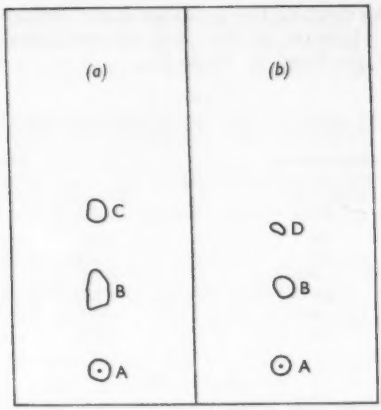

Fig. 2. Detection of octanol and thymol in presence of a large excess of ethanol: (*a*) chromatogram for mixture of 100 mg of octanol and 100 ml of 10 per cent. ethanol; (*b*) chromatogram for mixture of 1 litre of water, 1 ml of ethanol and 5 mg of thymol. A = 3,5-dinitrobenzoic acid; B = ethanol; C = octanol; D = thymol

of the 3,5-dinitrobenzoates can even be separated from large amounts of the unchanged acid for melting-point determinations, saponification, etc. The esters can be hydrolysed, and the original hydroxyl compounds can then be subjected to other chromatographic methods or to chemical procedures.

The locations of the spots are most suitably measured by comparison with a standard substance, as R_F values are influenced by many factors in this kind of adsorption chromatography.

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The Determination of Zinc in Agricultural Materials by Atomic-absorption Spectrophotometry

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An investigation of various aspects of the determination of zinc in agricultural materials by atomic-absorption spectrophotometry is described. Satisfactory recoveries for zinc were obtained from fertilisers, soils, soil extracts and plants. The method is rapid and accurate, and with the apparatus used the limit of sensitivity is 0·025 p.p.m. of zinc. Coefficients of variation of 2 to 3 per cent. are obtained over the range 0·3 to 8·0 p.p.m. of zinc.

The use of atomic-absorption spectrophotometry for determining zinc in plant material was described by David,[1] who found that no interference was caused by the other elements present in plants and considered that the technique was superior to chemical and polarographic

methods for determining this element. Gidley and Jones[2] have reported the successful application of atomic-absorption spectrophotometry to the determination of zinc in a variety of metallurgical materials. They investigated many elements for interference effects and found that only silicon caused depression of absorption. In this laboratory, atomic absorption has been successfully used for the past 3 years for the routine determination of zinc in a variety of agricultural materials, and the results are presented in this paper.

Measurement of Zinc Absorption

Apparatus and Methods—

The general arrangement of the apparatus and the method of measurement were described previously for the determination of magnesium.[3] Two types of hollow-cathode lamp have been used. One (kindly supplied by Mr. A. Walsh, C.S.I.R.O., Melbourne, Australia) had a zinc cathode and was operated at a current of about 10 to 12 mA; the other (obtained from Hilger & Watts Ltd., London) had a brass cathode and was operated at about 40 to 45 mA.

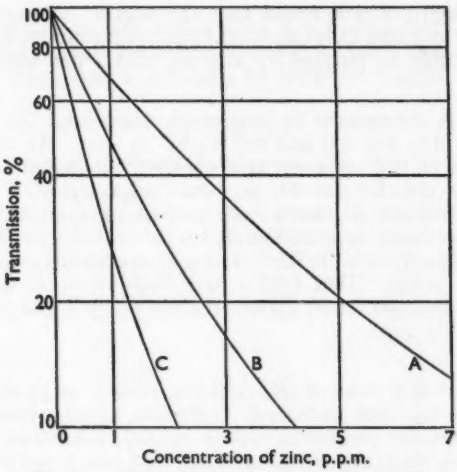

Fig. 1. Absorption for zinc measured at 2138·6 A: curve A, in aqueous solution; curve B, in 40 per cent. acetone; curve C, in isobutyl methyl ketone

With both types of lamp, a "warming-up" period of 30 to 60 minutes was necessary before maximum intensity was attained. Both lamps gave results similar in sensitivity and reproducibility, but that having the brass cathode had the longer life. The operating currents used were the minimum necessary to ensure that full-scale readings were attainable, and, with both lamps, reduction of current to below the values mentioned above led to a rapid decrease in intensity.

The burner used provided a flame 12 cm in length and has been described by Clinton[4]; the atomiser was of the Lundegårdh type, modified to aspirate solutions directly from a beaker. The zinc line at 2138·6 A was used for all measurements, this being the only line in the zinc spectrum that gives measurable absorption.

Sensitivity—

The sensitivity attainable with this equipment when aqueous solutions were sprayed into an air - acetylene flame is shown by curve A in Fig. 1. Linearity and sensitivity depend to some extent on the particular lamp used, and this curve is an approximate average of results obtained over 3 years, which in the extremes gave transmissions of 20 per cent. for 3 and 7 p.p.m. of zinc. The sensitivity is about four times that reported by Gidley and Jones and about ten times that reported by David, and, although most of this increase in sensitivity is due to the longer flame used, some contribution may have come from the atomiser. When a coal-gas flame is used, the sensitivity is increased by about 20 per cent., but there is no increase with a fuel-rich flame, indicating that the dissociation of zinc into atoms is virtually complete.

USE OF ORGANIC SOLVENTS—

The effect of spraying an organic rather than an aqueous solution has been discussed in detail elsewhere.[5] Curves B and C in Fig. 1 were plotted from the results obtained with solutions made up in 40 per cent. acetone and isobutyl methyl ketone, respectively. Ammonium pyrrollidine dithiocarbamate was used to form a complex with the zinc before extraction into the latter solvent, as this complex is readily soluble in esters and ketones, which are particularly suitable for spraying into flames.

The effect of pH on the extraction has been investigated. To a 10-ml portion of each of a series of sodium acetate - hydrochloric acid solutions, containing from 1 to 20 μg of zinc, were added 1 ml of a 1 per cent. aqueous solution of amonium pyrrollidine dithiocarbamate and 10 ml of isobutyl methyl ketone. The mixture was shaken, and, after the layers had separated, the zinc content of each layer was determined. The percentages of zinc extracted into the organic layer from solutions of pH 1·5, 2·1 and 2·5 to 5·0 were, respectively, 67, 95 and 100.

Somewhat surprisingly, it was found that the organic solvents used (isobutyl methyl ketone, ethyl pentyl ketone and ethyl acetate) frequently contained traces of zinc; however, these solvents could readily be purified by washing with 2 per cent. hydrochloric acid.

REPRODUCIBILITY—

Reproducibility was determined by repeatedly measuring the absorptions of a series of solutions containing 0·4, 1·6, 4·0 and 8·0 p.p.m. of zinc. At these concentrations, the coefficients of variation of the apparent zinc content, calculated from twenty-one sets of measurements, were 3·1, 2·5, 1·8 and 3·0 per cent., respectively.

With adequate control over hollow-cathode current, photomultiplier voltage, air pressure and flow of gas, reproducibility depends largely on the stability of the hollow-cathode lamp. In my experience, the above results are fairly average; on occasions, better have been obtained, and, with some lamps, worse. That Gidley and Jones obtained a coefficient of variation of 3·5 per cent. after integration for 30 seconds reflects the fact that at present not all hollow-cathode lamps are equally good.

INTERFERENCE—

David's observation[1] that none of the elements present in plant digests interferes with the determination of zinc has been confirmed. Likewise, to judge from the results in Table II (p. 533), none of the elements present in soil or fertiliser solutions causes any interference, provided always that the total concentration of salt and acid is not sufficiently great to alter the physical properties of the solution to an extent such that atomisation is affected. The

TABLE I

EFFECTS OF DIFFERENT ACIDS ON ZINC ABSORPTION

Zinc present, p.p.m.	Galvanometer reading for solution in— hydrochloric acid	sulphuric acid	nitric acid	perchloric acid
0·0	100	100	100	100
0·3	88·3	88·5	88·5	87·0
0·6	78·5	79·0	79·0	79·0
1·2	64·0	64·5	64·7	64·8
3·0	35·5	35·5	35·5	34·5
6·0	18·5	18·5	18·5	18·3

plant-digest solutions used contained about 2 ml of 72 per cent. perchloric acid in 20 ml of solution; this was sufficient to cause results for zinc to be low by some 5 to 7 per cent. unless standards containing approximately the same amount of acid were used.

The effects of various acids on measurement of the zinc absorption were tested by using a series of solutions containing up to 6 p.p.m. of zinc, prepared in 0·5 N hydrochloric, sulphuric, nitric and perchloric acids. For these experiments the apparatus was set to give 100 per cent. transmission when distilled water was sprayed into the flame. The results obtained with an air - acetylene flame are shown in Table I, from which it can be seen that no acid itself caused any absorption and that, within the experimental error of reading the galvanometer, zinc absorption was the same in the four acids. Similar results were obtained with air - coal-gas and air - propane flames.

The observation by Gidley and Jones[2] that an absorption band was produced when halogen acids were sprayed into the flame and interfered with measurement of zinc absorption led to experiments in which it was established that the presence of hydrochloric acid caused no difference in the absorption at 2138 A under a wide variety of flame conditions and in presence of a number of compounds that could conceivably occur in flames in some circumstances. It was concluded that Gidley and Jones's results were caused by contamination from a brass burner giving rise to the zinc line at 2138 A and the copper lines at 2165, 2178 and 2183 A, which have been shown[6] to absorb strongly. This conclusion was reached by Gidley and Jones in a Note[7] that appeared after this paper had been submitted.

Analytical Applications

The methods used and the results obtained for the determination of zinc in various agricultural materials are briefly described below.

Fertilisers—

A suitable weight of sample was boiled with 3 N hydrochloric acid, the solution was evaporated to dryness, the residue was dissolved in 0·5 N hydrochloric acid, and this solution was diluted to 100 ml. Recoveries and results for different weights of sample are shown in Table II.

Table II

Zinc found in fertilisers, soil and soil extract

Sample	Amount of sample taken	Zinc added, μg	Zinc found per g of sample, μg	
Fertiliser A*	1·0 g	—	294	Average 293
	0·25 g	—	292	
	0·25 g	100	692	(Expected 693)
	0·25 g	200	1084	(Expected 1093)
Fertiliser B†	1·0 g	—	485	Average 487
	0·25 g	—	488	
	0·25 g	100	892	(Expected 887)
	0·25 g	200	1280	(Expected 1288)
Soil	4·0 g	—	84‡	
	2·0 g	—	89	Average 90
	1·0 g	—	90	
	1·0 g	100	188	(Expected 190)
	1·0 g	200	295	(Expected 290)
Soil extract	10 ml undiluted	—	10·3	Average 10·5
	5 ml diluted to 10 ml	—	10·4	
	2·5 ml diluted to 10 ml	—	10·8	
	5 ml diluted to 10 ml	6·25	11·4	(Expected 11·5)

* Superphosphate.

† Proprietary fertiliser consisting essentially of lime, superphosphate, limonite and small amounts of various salts, including zinc sulphate.

‡ Low result probably due to decrease in efficiency of atomiser.

Soils—

Various amounts of soil were digested with nitric and perchloric acids, silica was removed with hydrofluoric acid, and the solution was evaporated to dryness; the residue was dissolved in 5 per cent. perchloric acid, and the solution was diluted to 50 ml. Results for soils are also shown in Table II. The low result for the most concentrated solution was probably caused by a decrease in the efficiency of the atomiser owing to the high salt content. A similar effect occurred when these solutions were analysed for copper by the same method. For most soils, 1- to 2-g are sufficient.

Soil extracts—

A 2-g sample of a peat soil was shaken with 20 ml of a 1 per cent. aqueous solution of disodium ethylenediaminetetra-acetate (a commonly used extractant for "available" zinc) for 2 hours, and the mixture was then filtered. Various aliquots of the filtrate were diluted to 10 ml, and the solutions were analysed for zinc; standards made up with 0·1 N hydrochloric acid were used. The results are also shown in Table II.

PLANTS—

Triplicate samples of various sizes were digested with 20 ml of a nitric - perchloric acid mixture (17 ml of concentrated nitric acid and 3 ml of 72 per cent. perchloric acid). After the solutions had cleared, each was concentrated to about 2 ml, transferred to a 20-ml calibrated flask, diluted to the mark with water and filtered. The filtrates were analysed for zinc, standards made up in 10 per cent. perchloric acid being used. The results are shown in Table III; each represents a single determination, and sample heterogeneity and chance contamination may have contributed to the variation between replicates.

TABLE III

ZINC FOUND IN APPLE AND GRASS

Sample	Weight of sample taken, g	Zinc added, μg	Zinc found per g of oven-dried sample, μg	
Apple	1·0	—	2·8, 3·1, 2·9 (mean 2·93)	Average 3·00
	2·0	—	3·1, 3·1, 3·0 (mean 3·07)	
	1·0	4·8	7·7, 7·4, 7·9 (mean 7·7; expected 7·8)	
Grass	0·5	—	42·0, 39·0, 40·0 (mean 40·3)	Average 39·9
	1·0	—	39·5, 40·5, 39·5 (mean 39·8)	
	2·0	—	39·8, 40·5, 38·8 (mean 39·7)	
	1·0	37·5	76·5, 78·0, 78·3 (mean 77·6; expected 77·4)	

CONCLUSION

The determination of zinc by atomic-absorption spectrophotometry compares more than favourably with other methods in simplicity, speed, reproducibility and accuracy. In a recent review of chemical methods for determining trace amounts of zinc, Margerum and Santacana[8] stated that their preferred method had an operating range of 3·3 (coefficient of variation 6·1 per cent.) to 32·7 μg of zinc (coefficient of variation 1·93 per cent.). The determination of zinc by neutron-activation analysis has also been described[9,10]; with this technique the ultimate sensitivity was 0·04 μg of zinc, and reliable determinations were possible on samples containing 0·3 μg of zinc.

In the proposed method, the limit of sensitivity (99 per cent. transmission) for an aqueous solution is 0·025 p.p.m. of zinc, which, when 2 ml of solution are used for a determination, amounts to 0·05 μg, and determinations of reasonable accuracy (coefficient of variation of, say, 5 per cent.) can be carried out on a solution containing 0·2 p.p.m. of zinc, *i.e.*, 0·4 μg per 2 ml. When it is convenient to extract the zinc into an organic solvent, these figures are decreased to about one-fifth.

I thank Miss V. O. Marnie Wright for assistance with the experimental and analytical work.

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Micro-quantitative Analysis by the Zone-Strip Technique

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The application of solution in a zone across a narrow strip of chromatography paper restricts the width of the loaded area on the developed strip. The length of the developed zone is proportional to the logarithm of the zone content, but this relationship holds only below a certain maximum concentration, depending on the nature of the compound. Calibration graphs were plotted from the results for analytical-reagent grade compounds. The graphs obtained for several compounds were adequate for assaying solutions containing 1 mg of the compound per ml. The technique may be of particular value for analysing biological fluids.

THE use of paper chromatography for micro-quantitative analysis has not yet attained a high degree of accuracy. Fisher, Parsons and Morison[1] reported that the area of a round or ovoid spot increased with the logarithm of the spot content. For ovoid regular spots, the length is proportional to the logarithm of the spot content.[2] Brown and Marsh[3] increased the sensitivity of evaluating low concentrations of light-absorbing materials on paper-strip chromatograms by using a special attachment designed for use with a monochromator incorporating a stabilised light source.

However, round or ovoid spots occur infrequently on developed chromatograms, and, because of this, we applied solutions in zones 2 mm wide near the tops of strips 0·5 cm wide. When these strips were developed, the width of a loaded area was restricted to 0·5 cm and its length was influenced by the content of the zone; graphs of length of zone against logarithm of zone content were linear.

Experimental

The pure standard antibiotics used were generously offered by Pfizer & Co. Inc., U.S.A., Leo Pharmaceutical Products Trading Ltd., Denmark, Imperial Chemical Industries Ltd. and the Medical Research Council (International Standard penicillins), England. The rest of the compounds used were of analytical-reagent grade and were obtained from the British Drug Houses Ltd. or from E. Merck A.G., Darmstadt, Germany.

Chromatography of penicillins and tetracyclines—

For assaying the different penicillins (G, V, K, X, F and dihydro-F), sheets of Whatman No. 1 chromatography paper were soaked in a 30 per cent. solution of phosphate buffer (pH 6·2) and then cut longitudinally into strips 0·5 cm wide. Results were more accurate when narrower strips were used, but such strips were difficult to manipulate. At 8 cm from the end of each strip, two pencil lines were drawn 2 mm apart; the area between the two lines was found to absorb 0·00075 ml of liquid when a fine capillary tube was passed over it. Diethyl ether saturated with water was used as mobile phase, and saturation of the atmosphere in the chromatographic tank with ether and water vapours resulted in improved resolution of the different penicillins. The mobile phase was allowed to descend through the strips for 40 cm, and the strips were then removed, dried and subjected to any of the six treatments described below.

(*i*) They were sprayed with an aqueous 2 per cent. solution of ferric chloride.

(*ii*) They were sprayed with a 1 per cent. solution of iodine and then with a solution of starch.

(*iii*) They were sprayed with the iodine solution and then subjected to ultra-violet light.

(*iv*) The strips after treatment (*i*) were washed in a stream of tap-water until free from excess of the ferric salt, dried and then sprayed with a 20 per cent. solution of potassium ferrocyanide or with a 2 per cent. solution of sodium thiocyanate.

(*v*) They were sprayed with a 1 per cent. solution of potassium permanganate.

(*vi*) They were sprayed with an ammoniacal solution of silver nitrate and then set aside for 6 hours at 60° C.

The strips used for assaying the different tetracyclines were soaked in a 20 per cent. solution of phosphate buffer (pH 3·5) and dried in air, and the solutions were applied to 2-mm zones; the strips were then developed with ethyl acetate or aqueous butyl alcohol.

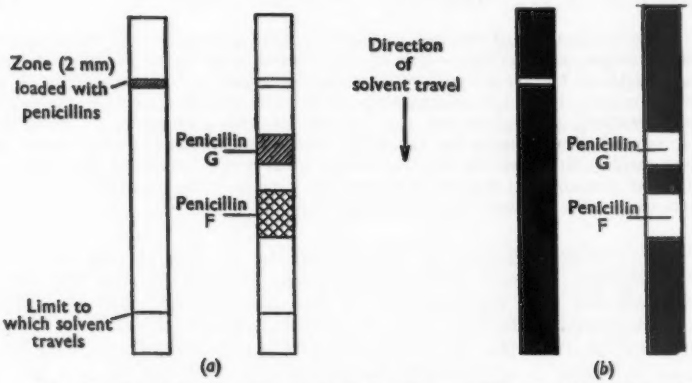

Fig. 1. Reproduction of strips loaded with penicillins G and F and treated with (*a*) solutions of iodine, ferric chloride and potassium ferrocyanide and (*b*) solutions of iodine and starch. The left-hand strip of each pair is undeveloped

The developed strips were sprayed with a 1 per cent. solution of alkaline potassium permanganate or, preferably, a 4 per cent. solution of sodium hydroxide and then subjected to ultra-violet light.

For comparison, antibiotics were also assayed by biological methods. The procedure used for the penicillins was a slightly modified version of that described by Goodall and Levi.[4] The lengths of the zones on developed strips were measured with a travelling microscope.

CHROMATOGRAPHY OF OTHER COMPOUNDS—

Sugars, amino acids, ascorbic and other organic acids and adrenalin were assayed on unbuffered strips of paper, with a butyl alcohol - acetic acid - water mixture (4:1:5) as developing solvent. The loaded zones were made visible by spraying them with ethanolic

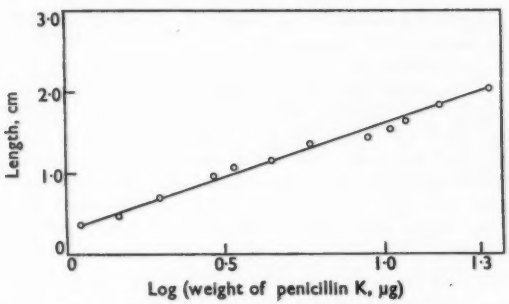

Fig. 3. Graph showing relationship between length and logarithm of content of zone containing penicillin K

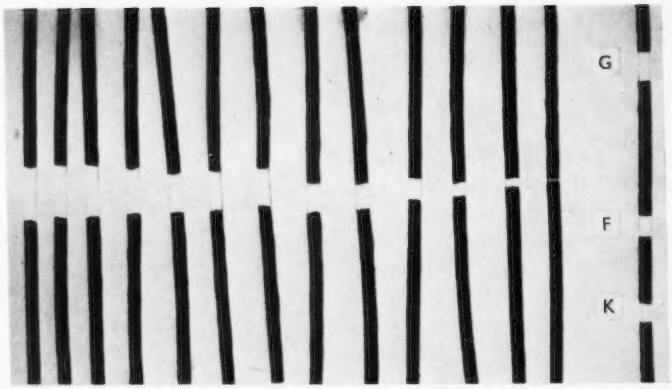

Fig. 2. Developed strips for penicillins after treatment with solutions of iodine and starch

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solutions of ninhydrin (for amino acids) or *p*-bromophenol (for other organic acids). For sugars, Harris and MacWilliam's[5] diphenylamine - aniline - phosphoric acid - water reagent was used. Aqueous solutions of 2,6-dichlorophenol endophenol and ferric chloride were used to define the areas containing ascorbic acid and adrenalin, respectively.

Results and discussion of the method

The different reagents applied to the strips containing penicillins induced marked colour contrasts (see Figs. 1 and 2), and results were in good agreement with those found by bioassay. Table I shows the effects produced by the various reagents. The relationship between length and logarithm of content of zone for penicillin K is shown in Fig. 3.

We favour treatment of the penicillin-loaded strips with iodine solution, ferric chloride solution and then ultra-violet light; this gives an obvious colour contrast, and the zones have sharp edges. Application of ferrocyanide solution preserves the colour contrast for several months. This procedure can be successfully applied to solutions of penicillin-producing moulds, even in presence of some natural products, *e.g.*, corn-steep liquor or molasses.

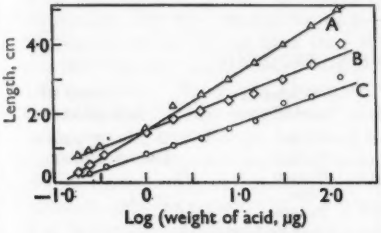

Fig. 4. Curve A, tryptophan; curve B, cystine; curve C, glutamic acid

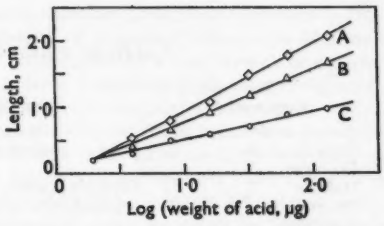

Fig. 5. Curve A, aconitic acid; curve B, citric acid; curve C, malic acid

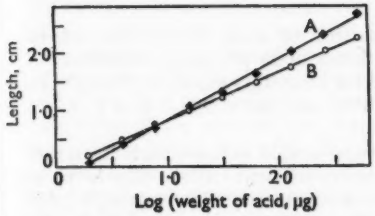

Fig. 6. Curve A, succinic acid; curve B, gluconic acid

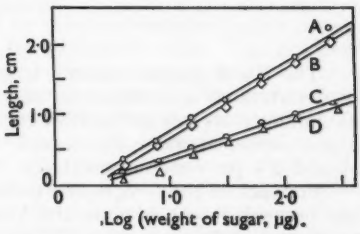

Fig. 7. Curve A, fructose; curve B, maltose; curve C, sucrose; curve D, raffinose

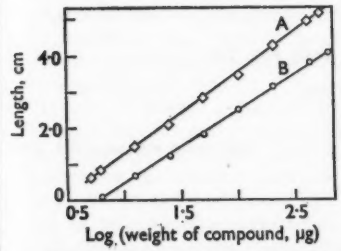

Fig. 8. Curve A, adrenalin; curve B, ascorbic acid

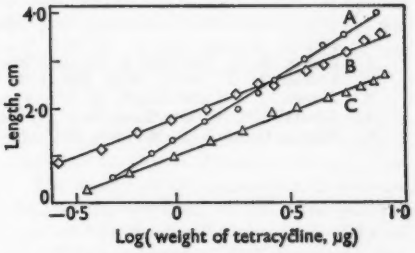

Fig. 9. Curve A, chlortetracycline; curve B, oxytetracycline; curve C, tetracycline

Figs. 4 to 9. Graphs showing relationships between lengths and contents of zones containing various compounds

TABLE I

COLOURS PRODUCED WITH PENICILLINS BY DIFFERENT REAGENTS

Treatment No.*	Colour of penicillin-loaded zone	Colour of unloaded area
(*i*)	Buff, with hard texture	White or pale buff
(*ii*)	White	Dark blue
(*iii*)	Shiny white	Dark shiny violet
(*iv*)†	Deep blue	White or pale blue
(*iv*)‡	Deep red	White or pale red
(*v*)	White	Violet
(*vi*)	White	Brown or black

* See pp. 535–536.
† Sprayed with potassium ferrocyanide solution.
‡ Sprayed with sodium thiocyanate solution.

The colours produced when developed strips containing other compounds are sprayed with different reagents are listed in Table II, and the relationships between length and logarithm of content of zone for these compounds are shown in Figs. 4 to 9.

TABLE II

COLOURS PRODUCED WITH VARIOUS COMPOUNDS

Compound	Spray reagent	Colour of loaded zone	Colour of unloaded area
Amino acids	Ninhydrin	Pink	White or pale red
Organic acids ..	*p*-Bromophenol	Yellow	Blue or violet
Fructose	Diphenylamine, aniline and phosphoric acid	Brown	White
Maltose		Blue	
Sucrose		Brown	
Raffinose		Grey or blue	
Ascorbic acid ..	2,6-Dichlorophenol endophenol	White	Pink or pale violet
Adrenalin	Ferric chloride	Dark green changing to brown	Pale buff
Tetracyclines	Sodium hydroxide	Yellow	White or violet in ultra-violet light

The linear graphs relating length of zone to logarithm of zone content are adequate for determining concentrations of about 1 mg of the compounds per ml of solution. The maximum errors calculated when these graphs were used for assaying penicillins, tetracyclines, organic acids, amino acids, sugars, adrenalin and ascorbic acid were 2·2, 1·6, 1·1, 1·7, 2·8, 1·3 and 2·4 per cent., respectively.

For each of the compounds tested, there is a maximum weight below which the relationship between length of zone and logarithm of zone content is linear; above this level, such a relationship does not exist. Within a narrow range of concentrations linear graphs relating length of zone and zone content could be obtained. Such graphs were excellent for assaying the compounds tested, but were of limited application. Further work on the various factors influencing this technique and its applications is in progress.

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Notes

THE MICRO-DETERMINATION OF CARBON AND HYDROGEN IN FLUORINE-CONTAINING ORGANIC COMPOUNDS

WHEN fluorine-containing compounds are analysed for their carbon and hydrogen contents, special precautions must be taken to avoid errors arising from the presence of fluoride ion. Low values can be obtained for the carbon contents of fluorocarbons because they resist decomposition, owing to the stability of the fluorine - carbon bond. Generally, results are high because some silicon tetrafluoride is formed by interaction between the fluoride ions and the silica of the combustion tube; this tetrafluoride is retained by the absorbent in the carbon dioxide absorption tube. These observations are related to slow-combustion methods, in which combustion is carried out with oxygen flowing at 4 to 10 ml per minute and the products of decomposition pass through layers of copper oxide or are in contact with a catalytic surface and silver in the hot zone of the combustion tube, or both.

The constituents of the products of oxidation of fluorine-containing compounds are not known exactly, but it is reasonable to assume that elemental fluorine is the initial product. This assumption is based on the fact that the theoretical amount of elemental chlorine is obtained when a chlorine-containing compound is decomposed by combustion in a fast stream of oxygen in an empty tube.[1] Similarly, bromine is the main product from a bromine-containing compound, and certainly only iodine is obtained from combustion of an iodine-containing substance. Some hydrofluoric acid must also be produced to account for the formation of silicon tetrafluoride. This reaction can be quantitative, as shown by Milner[2] and Clark,[3] who have used the reaction to determine fluorine by absorption of the terafluoride in water and subsequent titration of the hydrofluoric acid produced. Under normal conditions of combustion, some of the silicon tetrafluoride is decomposed and silica is deposited in the combustion tube, as found by Belcher and Goulden,[4] who have studied the determination of carbon and hydrogen in fluorine-containing compounds. The elemental fluorine and its hydride will then be retained by the hot silver forming part of the combustion-tube filling.

Belcher and Goulden[4] retained the silicon tetrafluoride by means of sodium fluoride heated to 270° C, the reagent being packed in the "beak" end of the combustion tube. The hot zone contained a roll of platinum foil maintained at 800° C. Granulated magnesium oxide was used by Throckmorton and Hutton,[5] who replaced part of the copper oxide packing at the front end of the tube with a 35- to 40-mm layer of the reagent; the temperature of the hot zone was 775° C. Fluorocarbons and chlorofluorocarbons were analysed without trouble, whereas Belcher and Goulden found it necessary to mix samples of fluorocarbons with a known weight of a hydrogen-containing organic compound, *e.g.*, benzoic acid. The carbon content of the sample was calculated by deducting the theoretical amount of carbon derived from the weight of the additive. Magnesium oxide has also been recommended by McCoy and Bastin,[6] who placed a 50-mm layer of the reagent at each end of the copper oxide layer, which was heated at 900° C. Rush, Cruikshank and Rhodes,[7] however, found that the magnesium oxide became inactive after a few determinations, and this they attributed to prolonged heating or exhaustion of the reagent. These workers recommended the use of a magnesium - aluminium compound, $3MgO.Al_2O_3$, maintained at 950° C, together with lead sesquioxide at 450° C. Short layers of silver gauze were distributed throughout the combustion zone, where the temperature was about 450° C. Lead dioxide at 180° C was also included in the tube filling to retain oxides of nitrogen arising from the combustion of nitrogen-containing compounds.

It appears from the observations of several workers that, for fluorocarbons, little of the sample is decomposed during the initial stages of the combustion. Most of the sample is vaporised and passes virtually unchanged into the hot zone, where complete or partial decomposition occurs, depending on the thermal stability of the compound. The extent of decomposition is also dependent on the temperature of the hot zone and, equally, on the efficiency of the tube packing (either in its oxidative capacity or catalytic effect) or on its affinity for fluorine ions. McCoy and Bastin,[6] for example, found that values for carbon were less accurate ($\pm 0{\cdot}5$ per cent.), but Macdonald[8] found that, when a platinum catalyst was used in place of the copper oxide, results had the required accuracy (within $\pm 0{\cdot}3$ per cent.). The presence of water, even in trace amounts, has a pronounced effect on the course of combustion of fluorocarbons. Freier, Nippoldt, Olson and Weiblen[9] have extended the silicon tetrafluoride method of determining fluorine to include

the determination of carbon. For this, they used a tube packed with alternate layers of quartz chips and platinum gauze, which was maintained at about 1200° C; moist oxygen was passed through the tube at 25 ml per minute.

Application of Belcher and Ingram's[10] rapid-combustion method has only recently been extended to the determination of fluorine-containing compounds. Wood[11] has shown that fluoro-organic compounds (fluorocarbons were not tested) could be analysed within the accepted limits of accuracy by using the method developed by Belcher and Goulden[4] (absorption of the silicon tetrafluoride by means of sodium fluoride). The reagent was placed in a tube attached to the exit of the baffle-chamber combustion tube and heated to 270° C. The combustion tube contained the customary plug of quartz wool and roll of silver gauze.

Some years ago, I had to determine the carbon contents of some specimens of polytetrafluoroethylene. The determinations were carried out by the rapid-combustion method,[10] and it was found that the values for carbon were within the accepted limits of accuracy, and the hydrogen contents, which should have been negative, were not more than 0·1 per cent. No special precautions were taken to remove fluorine, other than by the roll of silver gauze normally present in the exit part of the combustion tube. Rush, Cruikshank and Rhodes[7] reported that when polytetrafluoromethane was analysed by the conventional combustion procedure, correct values for carbon were obtained, suggesting that the fluorine was completely retained by the silver packing. In view of these separate observations, further experiments have been carried out to determine the fate of the halogen when other fluorine-containing compounds are analysed by the rapid-combustion method. It has not been possible to study the efficiency of the rapid method for fluorocarbons, owing to the lack of suitable test compounds of the type known to cause trouble. It is hoped to continue the experiments when such test compounds become available.

Experimental

The combustion experiments were carried out with a modified version of Belcher and Ingram's rapid-combustion unit. The baffle-chamber combustion tube was mounted horizontally, and the combustion heater was a small electrically heated furnace, 65 mm in length, which was moved forward by a motor-driven mechanism. The speed of travel was controlled by means of coarse and fine resistors in the motor circuit, and manual movement of the furance was simulated by controlling the motor current with an energy regulator (Sunvic TYC). This arrangement permitted a wide range of combustion times. In this work, the speed of the heater was adjusted to give a 5-mm movement in 5 seconds, and the stationary time was 22·5 seconds; the sample boat was 40 mm from the main furnace. The total time of combustion was 8 minutes, and, when the heater had reached the main furnace, it was left in position for a further 7 minutes, during which combustion was completed and the products formed were swept out. The flow of oxygen was 50 ml per minute. In the various experiments described below, the inlet of the baffle chamber contained the customary plug of quartz wool, and the chamber was maintained at 900° ± 20° C. The water and carbon dioxide were collected in Flaschenträger absorption tubes packed with suitable reagents; when nitrogen-containing compounds were examined, an absorber[10] containing manganese dioxide was connected between the two absorption tubes to retain the oxides of nitrogen formed.

Retention of fluorine by silver—

Weighed samples (3 to 4 mg) of trifluoromethylbenzoic acid were combusted in the apparatus, a roll of silver gauze, 160 mm long, being inserted in the exit tube of the baffle chamber. Part of this roll (about 70 mm) was heated to 550° ± 10° C; the remainder reached to the "beak" of the exit tube in the normal way. Typical results are shown in columns A of Table I and indicate that, possibly, some silicon tetrafluoride was formed during the combustion and retained in the carbon dioxide absorption tube. However, when the nitrogen oxides absorber was connected between the absorption tubes, the values for carbon were within the limits of error, as shown. A small white deposit was also formed in the inlet connecting tube of the water-absorption tube; this was presumably silicon dioxide arising from decomposition of the tetrafluoride compound. The weight of the deposit was always less than 0·05 mg. Assuming that the theoretical amount of carbon dioxide had been obtained and that the excessive weight was related to silicon tetrafluoride, calculation showed that about 50 per cent. of the available fluorine had been collected as that compound.

Confirmation of the formation of silicon tetrafluoride was obtained in another experiment. The products of combustion were dried by passage through a water-absorption tube attached to

the exit of the combustion tube and then passed through a bead absorber[12] containing about 5 ml of water. The hydrofluoric acid formed according to the reaction[9]—

$$SiF_4 + 2H_2O \longrightarrow 4HF + SiO_2$$

was titrated with 0·01 N sodium hydroxide. A white band of silica was deposited in the bead absorber at the point where the gas first came into contact with the moistened beads. The amount of alkali consumed was equivalent to approximately 50 per cent. of the available fluorine.

RETENTION OF FLUORINE BY MAGNESIUM OXIDE—

Weighed samples (3 to 4 mg) of trifluoromethylbenzoic acid were analysed as described above, but the silver in the exit tube was replaced by a layer of 14-mesh granules of pure magnesium oxide. The layer of reagent (about 60 mm long) was positioned between 10-mm wads of silver wool in the part of the tube heated by the furnace. The remaining space up to the "beak" was occupied by a roll of silver gauze. The oxide was heated to 550° C in some tests, but this temperature was increased to 750° C in others. The results of these experiments are shown in columns B of Table I, together with some values found for trifluoroacetanilide, the nitrogen oxides absorber being included in the system. The results for *m*-trifluoromethylbenzoic acid show that no silicon tetrafluoride was carried over into the carbon dioxide absorption tube. There was no deposit in the inlet connecting tube of the water-absorption tube. Further, the reagent was efficient in retaining the fluorine compound at the lower temperature of 550° C.

RETENTION OF SILICON TETRAFLUORIDE BY MANGANESE DIOXIDE—

In this series of experiments, only silver was used to retain the fluorine combustion products, but, as the compound being examined was trifluoroacetanilide, the absorber containing manganese dioxide was connected between the water and carbon dioxide absorption tubes. Samples weighing 3 to 4 mg were taken for the analyses, and some results are shown in columns A of Table I. In distinct contrast to the previous analyses of *m*-trifluoromethylbenzoic acid, when only silver was used to retain fluorine, the values for carbon had the required accuracy (within $\pm 0{\cdot}3$ per cent. of the theoretical figure). In these experiments, the white deposit was found in the inlet of the water-absorption tube.

It was thus established that manganese dioxide at room temperature retains silicon tetrafluoride quantitatively, possibly according to the reaction—

$$MnO_2 + SiF_4 \longrightarrow MnF_4 + SiO_2$$

A further study of this absorption phenomenon is being carried out to determine the mechanism of the reaction.

RECOMMENDED TECHNIQUE FOR ANALYSING FLUORINE-CONTAINING COMPOUNDS

The values found for carbon and hydrogen indicated, at least for the two substances examined, that silicon tetrafluoride could be quantitatively retained within the combustion tube by means

TABLE I

CARBON AND HYDROGEN CONTENTS FOUND UNDER VARIOUS CONDITIONS

The values for hydrogen content listed under A were determined after deposited silica had been removed

Compound tested	A		B		C	
	Carbon content found, %	Hydrogen content found, %	Carbon content found, %	Hydrogen content found, %	Carbon content found, %	Hydrogen content found, %
m-Trifluoromethylbenzoic acid (carbon content, 50·54%; hydrogen content, 2·65%)	55·60	2·77	50·42	2·82*	50·70	2·80
	57·90	2·68	50·37	2·57*	50·50	2·52
	50·57	2·53†	50·72	2·85‡	50·73	2·52
	50·54	2·61†	50·55	2·60‡	50·46	2·68
Trifluoroacetanilide (carbon content 50·80%; hydrogen content, 3·20%)	50·50	3·22	50·84	3·15*	50·71	3·35
	50·66	3·12	50·83	3·20*	50·73	3·13
	50·93	3·25	50·95	3·23*	50·83	3·18
	—	—	50·65	3·35‡	50·84	3·22
	—	—	50·99	3·14‡	—	—

* Magnesium oxide maintained at 550° C.
† Absorber containing manganese dioxide inserted between absorption tubes.
‡ Magnesium oxide maintained at 750° C.

of magnesium oxide when the rate of flow of oxygen was 50 ml per minute. Manganese dioxide can also serve as an external absorbent for the tetrafluoride at room temperature, but then a slight error in the values for hydrogen can be expected because of the small amount of silica collected in the inlet tube of the water-absorption tube. The deposit can be easily removed before the absorption tube is weighed, but traces of the solid may be swept into the absorber, although no evidence of this was obtained.

Instead of the magnesium oxide being packed directly into the combustion tube, it was considered that it would be more convenient to place the reagent inside the roll of silver gauze, so that it could readily be changed when necessary without dismantling the apparatus. This simplified procedure was examined and found to provide an alternative to the packed-tube technique without a decrease in accuracy. A cylinder was made from a strip of 60-mesh silver gauze 160 mm long and about 40 mm wide, and this cylinder fitted snugly into the exit tube of the baffle chamber. A second strip of the gauze, 85 mm in length, was rolled round a piece of thick silver wire, which was bent into a hook to allow the prepared cylinder to be withdrawn from the tube. This roll was inserted in one half of the cylinder so that the hook projected outside. The remaining space in the cylinder was packed with 14-mesh granules of pure magnesium oxide, and the open end was pinched together to hold the reagent in the cylinder.

The prepared roll was inserted in the combustion tube and conditioned in a current of oxygen at its working temperature of 550° C until the blank values were satisfactory. This modified filling has proved reliable in the analysis of fluorine-, chlorine-, bromine-, iodine- and sulphur-containing compounds, but has not yet been used for the analysis of fluorocarbons. Some typical results for carbon and hydrogen are shown in columns C of Table I.

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DETECTION OF PYRAMIDON AND ANTIPYRIN WHEN PRESENT TOGETHER

This Note describes two tests for detecting pyramidon (amidopyrin; 4-dimethylamino-2,3-dimethyl-1-phenylpyrazol-5-one) when present together with antipyrin (phenazone; 2,3-dimethyl-1-phenylpyrazol-5-one)[1,2,3,4]; ceric sulphate, platinic chloride and mercurous nitrate solutions are used.

Method

Reagents—

All materials should be of the highest grade of purity obtainable.

Ceric sulphate solution, 2 per cent.—Prepared from the hydrated salt.

Platinic chloride solution, 10 per cent.

Mercurous nitrate solution, saturated.

Pyramidon - antipyrin solutions—A series of aqueous solutions containing various concentrations of both compounds.

Test A—

To about 1 ml of the pyramidon - antipyrin solution in an 80-mm × 8-mm test-tube are added 1 or 2 drops of the ceric sulphate solution; a violet colour is produced in the presence of pyramidon. By this reaction, 0·012 mg of pyramidon per ml of solution can be detected in the

presence of any amount of antipyrin that does not react with the ceric sulphate. (If ceric sulphate is present in excess, antipyrin produces a red-orange colour when heated.) After the reaction with pyramidon, 5 to 6 drops of the mercurous nitrate solution are added to the contents of the test-tube. If antipyrin is present, a grey or black spongy sediment is formed immediately or after a short time; this precipitate, when heated with concentrated nitric acid, produces a red colour. By this reaction, about 0·03 mg of antipyrin can be detected in 1 ml of solution containing any amount of pyramidon.

It was found that the sensitivity of the test for antipyrin could be increased by using a mixed reagent consisting of 2 or 3 drops of the 10 per cent. solution of platinic chloride added to 100 ml of the ceric sulphate solution. When this reagent was used, a precipitate was formed in the presence of 0·015 mg of antipyrin per ml of test solution after the mercurous nitrate solution had been added. A similar result was achieved with a mixed reagent consisting of about 0·5 ml of a 1 per cent. solution of auric chloride added to 100 ml of the ceric sulphate solution.

TEST B—

To about 1 ml of the pyramidon - antipyrin solution in a small test-tube is added 1 drop of the platinic chloride solution; if pyramidon is present, a violet-blue colour appears. The colour is formed immediately with solutions containing about 0·02 mg of pyramidon per ml, but more slowly (or immediately after being heated) when the concentration of pyramidon is about 0·01 mg per ml. Antipyrin produces no colour. However, when 5 or 6 drops of the mercurous nitrate solution are added, a grey or black sediment appears when antipyrin is present, even at the level of 0·012 mg per ml of solution.

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DIRECT TITRATION OF HYDROLYSABLE SULPHUR IN ORGANIC COMPOUNDS

As previously described, numerous sulphur compounds react with *o*-hydroxymercuribenzoic acid in alkaline solution to form soluble colourless sulphides having the structure *R*HgSHg*R*; this reaction can be used for determining hydrolysable sulphur, and a procedure based on determining the unconsumed excess of *o*-hydroxymercuribenzoic acid after heating with the sample has been suggested.[1]

This Note describes the direct titration of hydrolysable sulphur with *o*-hydroxymercuribenzoic acid solution at room temperature, which has the advantages of being more selective and rapid. The procedure can be successfully used for the rapid determination of several sulphur compounds in the presence of each other, depending on their different rates of de-sulphuration.

When dithiocarbamates are titrated, the isothiocyanates formed undergo slow hydrolysis and so interfere with the determination. However, this can be avoided by removing the isothiocyanates from the aqueous solution with an organic solvent. Compounds such as β-aminoethyl- and β-hydroxyethylisothiocyanate undergo rapid transformation to the corresponding cyclic compounds, which are resistant to further de-sulphuration and do not interfere.

METHOD

PROCEDURE FOR PHENYL- OR ETHYLMONOTHIOCARBAMATE, DITHIOCARBAMATE, β-AMINOETHYL- OR β-HYDROXYETHYLDITHIOCARBAMATE, DIPHENYLTHIOUREA AND RUBEANIC ACID—

Add to the sample 5 ml of N sodium hydroxide, dilute to between 30 and 50 ml with water (or with methanol if the sample is insoluble in water), and titrate with 0·001 to 0·05 N *o*-hydroxymercuribenzoic acid. Thiofluorescein or dithizone can be used as indicator; with the former, the blue colour disappears at the end-point, and with dithizone the yellow colour changes to purple. The titration can be carried out at 20° to 25° C, but a temperature of 30° to 40° C is preferable.

PROCEDURE FOR ETHYL-, PHENYL- OR BENZYLDITHIOCARBAMATE—

Add to the sample 5 ml of N sodium hydroxide, dilute with water to 50 ml, add 5 to 20 ml of toluene, and titrate at 20° C with 0·001 to 0·05 N *o*-hydroxymercuribenzoic acid. Use thiofluorescein as indicator, shake well after each addition of titrant, and take the end-point as being when the blue colour disappears for at least 30 seconds.

Note that thiourea, ethylxanthate, *o*-phenylenethiourea, mercaptobenzothiazole, thiosulphate, thiocyanate and sulphite interfere not at all, or only slightly, with either titration. Recovery of the compounds tested was between 98·5 and 100 per cent., assuming that de-sulphuration was 50 per cent. for the dithiocarbamates and complete for the other compounds.

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ENZYMIC HYDROLYSIS OF PHOSPHOLIPIDS AS A MEANS OF DETERMINING EGG IN FOODS

WE have already outlined[1] a method of determining egg in food based on the hydrolysis of the phospholipids by lecithinase D followed by determination of the liberated choline. Originally, we prepared the enzyme concentrate from cabbage, but more recently we have had an opportunity of testing two samples of dried lecithinase D (one prepared from cabbage and the other from carrot) kindly supplied to us by C. F. Boehringer G.m.b.H., Mannheim, Germany (English agents, Messrs. Courtin and Warner Ltd., Lewes, Sussex). These samples represented enzyme concentrates that the suppliers propose to make available commercially.

TABLE I

CHOLINE CONTENTS FOUND IN EGG AND ICE-CREAM

Sample	Enzyme: Source	Enzyme: Amount used per determination, mg	Total choline found, %
Dried whole egg	Boehringer (from carrot)	10	1·51*
	Boehringer (from carrot)	30	1·62*
	Boehringer (from cabbage)	30	1·61*
	Cabbage†	200	1·63*
Ice-cream No. 1 (without egg)	Boehringer (from cabbage)	30	0·0125
Ice-cream No. 1 *plus* 2 per cent. of dried whole egg	Boehringer (from cabbage)	30	0·0466

* Choline content of dry matter.
† Extracted and dried as described previously.[1]

The results are shown in Table I, from which it is evident that a suitable proportion of either Boehringer lecithinase is 30 mg per determination. For the ice-cream, the choline derived from egg is, by difference, 0·0341 per cent; this corresponds to 2·1 per cent. of dried whole egg, whereas 2 per cent. was added.

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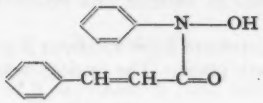
RAPID EXTRACTION AND SPECTROPHOTOMETRIC DETERMINATION OF VANADIUM WITH *N*-CINNAMOYL-*N*-PHENYLHYDROXYLAMINE

THE reagent *N*-benzoyl-*N*-phenylhydroxylamine, recommended by Shome[1] as superior to cupferron, has been widely used in analysis; it is an excellent reagent for the spectrophotometric determination of vanadium.[2,3,4] Tandon and Bhattacharyya[5] studied many *N*-acyl-substituted *N*-arylhydroxylamine derivatives to obtain more information on the factors influencing the

selectivity and sensitivity of their reactions with metal ions. These workers recommended *N*-cinnamoyl-*N*-phenylhydroxylamine (**I**) as a highly specific spot-test reagent for vanadium,



(**I**)

and this Note describes its use for the rapid spectrophotometric determination of milligram amounts of vanadium.

N-Cinnamoyl-*N*-phenylhydroxylamine has all the useful features of *N*-benzoyl-*N*-phenylhydroxylamine as a reagent for vanadium, but is superior in sensitivity of reaction. The molecular extinction coefficients of the violet complexes of *N*-cinnamoyl- and *N*-benzoyl-*N*-phenylhydroxylamine with vanadium are 6300 ± 50 and 4650 ± 50, respectively, at their wavelengths of maximum absorption (calculated on the basis of vanadium).

Method

Reagents—

N-Cinnamoyl-N-phenylhydroxylamine solution—The reagent as prepared in the laboratory[5] consists of pale-green crystals, melting-point 162° to 163° C, and is stable to heat, light and air. A 0·1 per cent. w/v solution in ethanol-free chloroform was used for the extractions; this solution, stored in a dark bottle, is stable for several days.

Ammonium vanadate solution, aqueous—The vanadium concentration was determined volumetrically with potassium permanganate solution.

Procedure—

Ensure that the vanadium in the sample solution is in the quinquivalent state by treating it with a few drops of a dilute solution of potassium permanganate until a faint pink colour persists. Transfer an aliquot of the treated sample solution, containing not more than 0·12 mg of vanadium, to a separating funnel, and add distilled water and hydrochloric acid until the volume is about 25 ml and the acidity is between 2·7 and 7·5 N. Add 8 to 10 ml of *N*-cinnamoyl-*N*-phenylhydroxylamine solution, shake the funnel vigorously, allow the layers to separate, and collect the chloroform layer in a 50-ml beaker containing about 1·5 g of anhydrous sodium sulphate. Wash the aqueous layer twice with 5-ml portions of chloroform to remove any residual violet colour, and add the washings to the contents of the beaker. Decant the violet solution from the beaker to a 25-ml calibrated flask, wash the adhering colour from the sodium sulphate crystals with small portions of chloroform, combine the washings with the main solution, and dilute to the mark. Measure the optical density against chloroform at 540 mμ in matched 1-cm silica or Corex cells with a Unicam SP500 spectrophotometer. Calculate the amount of vanadium corresponding to the optical density by reference to a calibration graph.

The 0·1 per cent. w/v solution of *N*-cinnamoyl-*N*-phenylhydroxylamine has practically no absorption at 540 mμ, so that the use of chloroform in the reference cell is satisfactory. All glassware must be free from ethanol.

Discussion of the Method

Colour reaction—

A solution of *N*-cinnamoyl-*N*-phenylhydroxylamine in chloroform reacts with quinquivalent vanadium in solutions 2 to 10 N in hydrochloric acid and produces violet-coloured extracts. The absorption spectrum of such an extract has a broad band at 530 to 550 mμ, the sides of the band being symmetrical. The chloroform used must be free from ethanol, otherwise the absorption spectrum of the complex is affected.[3] Many other organic solvents, *e.g.*, carbon tetrachloride, benzene, ethyl acetate and diethyl ether, could be used to extract the violet complex from the aqueous phase, but none of these was suitable for quantitative work, owing to the unfavourable distribution ratios for both reagent and complex.

There is no reaction between *N*-cinnamoyl-*N*-phenylhydroxylamine and quadrivalent vanadium.

Acidity—

For maximum colour development the concentration of acid in the aqueous phase should be between 2·7 and 7·5 N; most of our measurements were made at an acidity of about 4 N. Only

hydrochloric acid was suitable for adjusting the acidity, but the presence of other acids could be tolerated, provided that their concentration in the aqueous phase was less than 1 N.

STABILITY OF COLOUR—

Colour is extracted into the chloroform layer in about 2 minutes, and the extracts are stable for a few days if stored in a cool dark place. The optical densities of extracts stored for 1 week decreased by about 4 per cent.

CONCENTRATION OF REAGENT—

The optimum concentration of the reagent solution is 0·1 per cent. w/v, and maximum development of colour takes place when the molar ratio of vanadium to reagent is 1 to 10. In practice, approximately 70 mg of reagent were used for each 1 mg of vanadium, but larger excesses of reagent can be tolerated. The order in which the reactants are mixed is not critical.

ADHERENCE TO BEER'S LAW—

The coloured system obeys Beer's law at 540 mμ for concentrations of vanadium from 0·5 to 10 p.p.m. The optimum range for determining vanadium, based on Sandell's recommendations,[6] is approximately 1·5 to 5 p.p.m.

EFFECTS OF OTHER IONS—

The effects of various ions on the determination of vanadium with *N*-cinnamoyl-*N*-phenylhydroxylamine were almost the same as those found when *N*-benzoyl-*N*-phenylhydroxylamine was used.[3] Results obtained in the presence of ions commonly encountered in determinations of vanadium are shown in Table I; the ions were added as solutions prepared from analytical-reagent grade salts by West's procedure.[7] There was interference from the ions Ti^{4+} and Mo^{6+}, and the permissible limit of the former ion in the aqueous phase was 20 p.p.m. for each 1 p.p.m. of vanadium. Much higher concentrations of Mo^{6+} can be tolerated if several successive extractions are made with the reagent solution. The ions listed below did not interfere when the weight ratio of each to vanadium was 250 to 1—

Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Ce^{4+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Sr^{2+}, Th^{4+} UO_2^{2+}, WO_4^{2-}, Zn^{2+}, Zr^{4+}, acetate, borate, citrate, nitrate, perchlorate, phthalate, phosphate, sulphate and tartrate.

Higher weight ratios were studied for a few ions and were tolerable.

TABLE I

EFFECTS OF VARIOUS IONS

The concentration of vanadium present in each test was 0·093 mg per 25 ml

Ion present	Amount of ion added, mg	Optical density at 540 mμ	Amount of vanadium recovered, mg
None	—	0·460 ± 0·005	0·093
Al^{3+} added as $Al(NO_3)_3$	25	0·470	0·095
Co^{2+} added as $Co(NO_3)_2$	25	0·465	0·094
Cr^{3+} added as $Cr(NO_3)_3$ Cu^{2+} added as $Cu(NO_3)_2$ Fe^{3+} added as $Fe(NO_3)_3$	25	0·460	0·093
$[MoO_4]^{2-}$ added as $(NH_4)_2MoO_4$	15	0·430* 0·450†	0·087 0·091
Ti^{4+} added as $TiOCl_2$	5	0·400	0·081
WO_4^{2-} added as Na_2WO_4	20	0·455	0·092
Zr^{4+} added as $Zr(NO_3)_4$	25	0·460	0·093

* Normal extraction procedure.
† Two further extractions with 5-ml portions of reagent solution.

PRECISION—

The average optical density found for twelve replicate samples, each containing 0·093 mg of vanadium in a final volume of 25 ml, was 0·460; the mean deviation of these results was 0·005. The method is rapid, gives reproducible results and is not affected by wide variations in factors such as temperature, ionic strength and volume of the aqueous phase. The sensitivity of the reaction, calculated on the basis defined by Sandell,[6] is 0·008 μg of vanadium per sq. cm at 540 mμ.

We thank Principal Umadas Mukherjee and Dr. Sameer Bose for the generous provision of laboratory facilities. We also thank Dr. S. C. Bhattacharyya, Assistant Director, National Chemical Laboratory, Poona, for helpful discussion and interest in the work.

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Apparatus

APPARATUS FOR THE PREPARATION OF STANDARD GAS MIXTURES

Probably the greatest drawback to the gas-chromatographic analysis of mixtures of permanent gases is the need for calibrating the chromatograph. Calibration can be carried out very simply and rapidly if standard mixtures of gases contained under pressure in cylinders are available; however, such mixtures of certified composition are expensive and not readily available in most laboratories. We have found that an extremely simple apparatus incorporating calibrated glass syringes and a rubber football bladder can be used to prepare mixtures containing known amounts of permanent gases rapidly and with sufficient accuracy for routine work.

Description and use of apparatus

The apparatus is shown diagrammatically in Fig. 1. The 10- and 30-ml syringes are normal hypodermic syringes calibrated in 1- and 5-ml divisions, respectively. The 100- and 250-ml syringes are readily constructed from glass and are made gas-tight by the use of O-ring seals at

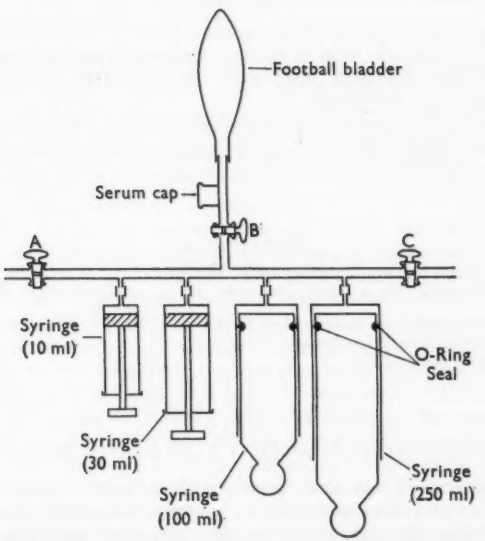

Fig. 1. Diagram of apparatus for preparing standard mixtures of gases

the ends of the plungers; they are calibrated in 25- and 50-ml divisions, respectively. Connections are by means of glass capillary tubing.

To prepare a gas mixture of known composition, the bladder is first evacuated via taps B and C; a suitable vacuum pump can be used or the plunger of the 250-ml syringe can be withdrawn several times, with manipulation of taps B and C. The latter method normally gives adequate evacuation of the bladder. The connecting lines are then flushed out via taps A and C (with tap B closed) with the pure gas to be introduced, and the volume of gas required is drawn into the appropriate syringe. Taps A and C are then closed, tap B is opened, and the desired volume of gas is transferred to the bladder. The procedure is repeated for as many components as are required. Very small amounts of a component may be injected directly into the bladder through the self-sealing rubber cap with a hypodermic syringe. The gases are finally mixed by manipulation of the bladder. The total volume of gas introduced should be such that significant pressure is not produced within the bladder. Samples for injection into the chromatograph can be withdrawn through the serum cap or, if sampling valves of the types described by Harvey and Chalkley[1] and Timms, Konrath and Chirnside[2] are used, the bladder can be detached and affixed to the sample inlet of the valve. It should be mentioned, naturally, that the material of the bladder must be completely inert, chemically and physically, to the gases used.

TABLE I

RESULTS FOUND FOR VARIOUS MIXTURES

Component	Concentration range, % v/v	Equation*	Standard error†	No. of mixtures
Hydrogen	0 to 1·75	H_2, % = 0·108h − 0·006	0·03% of H_2	8
	5 to 40	H_2, % = 0·172h − 0·32	0·2% of H_2	7
Carbon dioxide	15 to 35	CO_2, % = 0·276h + 0·34	0·3% of CO_2	6
Carbon monoxide	5 to 15	CO, % = 0·168h − 0·01	0·3% of CO	
Methane	1 to 6	CH_4, % = 0·161h − 0·18	0·12% of CH_4	
Oxygen	0 to 20	O_2, % = 0·967h − 0·05	0·3% of O_2	8
Nitrogen	0 to 1·5	N_2, % = 0·042h − 0·001	0·03% of N_2	11

* In these equations, h is the height, in millimetres, of the appropriate peak.

† Calculated from the expression $\sqrt{\frac{\Sigma (y - y')^2}{n - 1}}$, in which y is the concentration present (% v/v), y′ is the concentration calculated from the equation (% v/v) and n is the number of mixtures used.

RESULTS

The precision attained in preparing gas mixtures of known composition for calibration purposes is shown by the results in Table I; 1 ml of each mixture was injected into a Fisher Model 25 Gas Partitioner, the peak height for each component was measured, and the calibration equation for each component was then calculated.

The standard errors shown provide a good indication of the precision with which the mixtures were prepared, as the instrument gave reproducible results for several samples of the same mixture, and the response of the instrument was known to be linear over the small ranges of concentration used.

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AFRICAN EXPLOSIVES AND CHEMICAL INDUSTRIES LTD.
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Received *March 6th*, 1961

A SIMPLE AND INEXPENSIVE FRACTION COLLECTOR FOR CHROMATOGRAPHY

THE fraction collector described was built from readily available materials and components at a cost of approximately £5 and was assembled in a working time of 18 hours. Up to 100 fractions can be collected, and simple alternative devices for controlling collection by timed intervals or by volume are incorporated.

Construction was carried out in three stages, the first of which was the assembly of the motor and power pack. The motor used was a 12-volt, 1-amp ratchet motor obtained from Z and I Aero Services Ltd., 14 South Wharf Road, Paddington, London, W.2, which required only minor modifications. These were carried out as follows. The spindle for the ratchet wheel was removed and cut off ½ inch from the threaded end. A transverse cut was made across the cut end of the threaded piece to take a screwdriver. The upper guide hole for the spindle was drilled out to $\frac{5}{16}$ inch, and a No. 3 BA bolt 2 inches long was used as a spindle to carry the turn-table for connection to the ratchet wheel. The hollow stem of the ratchet-wheel assembly was threaded to take the No. 3 BA bolt. The ratchet wheel was then replaced, the shortened spindle being used as lower bearing, and the motor was bolted to a heavy wooden base.

The power pack was assembled from a 12-volt battery-charger transformer and a full-wave rectifier giving an output of 12 volts 1 amp. The ratchet-wheel assembly was already fitted with four "make-and-break" contacts operated by arms set at 90° intervals (see Fig. 1), and these were used to stop the fraction collector after 25, 50, 75 or 100 fractions had been collected.

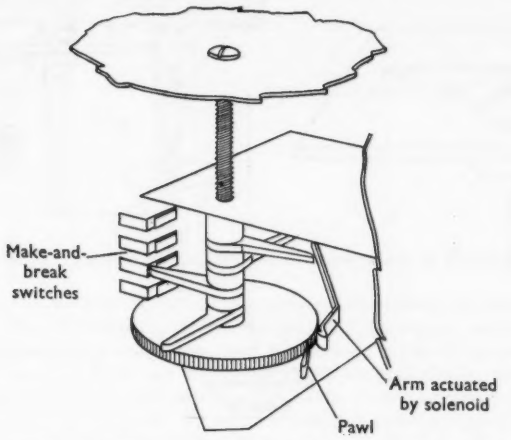

Fig. 1. Diagram of ratchet-wheel assembly showing "make-and-break" contacts

The second stage was the construction of the turn-table. The diameter chosen for the tubes was ½ inch, so that 3-inch × ½-inch or 5-inch × ½-inch tubes could be used. The centre-to-centre distance allowed for rimmed tubes ½ inch in diameter was 16 mm, which gave 25·5 cm as the radius from spindle to centre of tube; tubes of larger diameter would require a correspondingly larger turn-table. The turn-table (total radius 28 cm) consisted of an upper disc of 18 s.w.g. aluminium bolted to a ⅛-inch thick disc of hardboard for rigidity. After the positions of the tube centres had been marked on the aluminium disc the holes were drilled; the turn-table was then fixed on the 2-inch No. 3 BA bolt, which was screwed into the stem of the ratchet-wheel assembly. A friction brake acting on the rim of the turn-table was used to damp the movement.

Finally, the control devices were made. The time control was based on a synchronous electric-clock movement. A "wiping" terminal was attached to, but insulated by plastic sleeving from, the second hand and had a radius of 4 cm; the minute and hour hands were not used and were not attached to the movement. The current was carried by means of an overhead strip of aluminium to the centre where continuous contact was made with the "wiping" terminal. The "wiping" terminal was made to contact with copper rivets, set at 15, 30, 45 and 60 seconds, as it rotated. The rivets were ¼ inch in diameter and were sloped on the approach side and sharply cut off on the reverse side. Three Perspex shields were made to cover the rivets so that, by selective insulation, intervals of 15, 30 or 60 seconds could be obtained.

The volume-control device (see Fig. 2) was a balanced siphon, the movement of which was made to operate a simple switch consisting of a platinum wire, attached to the balance arm, making contact with a pool of mercury in a tube. The movement was restricted to ½ inch at the extremity. The balance arm, made of aluminium rod, was used as one terminal and the pool

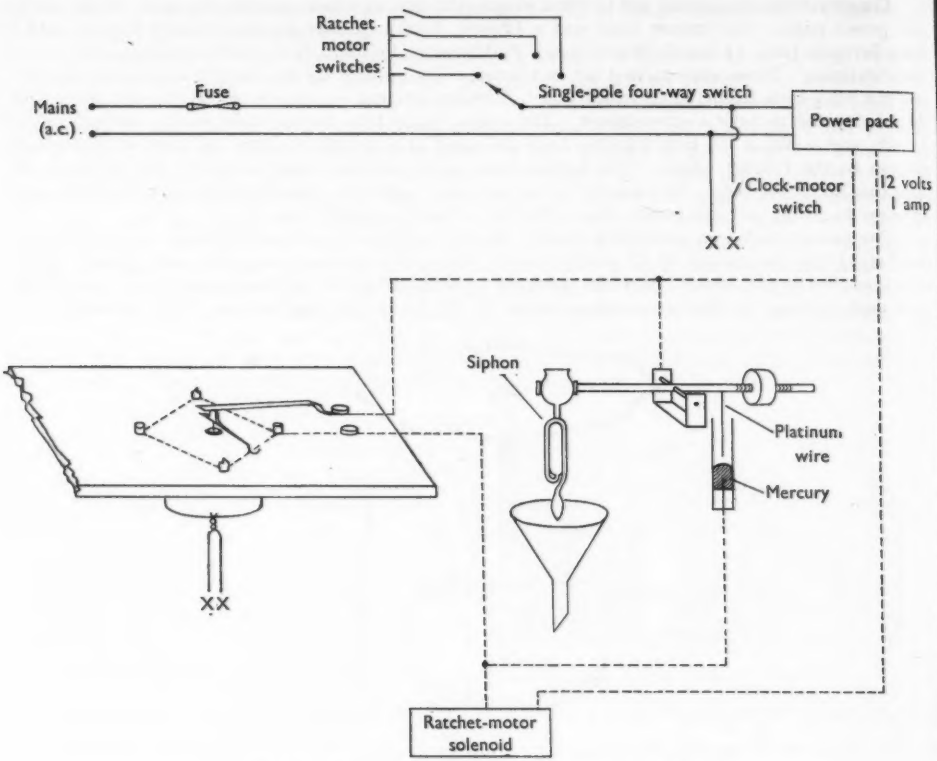

Fig. 2. Diagram of volume- and time-control devices

of mercury as the other. Contact of the switch was made when the siphon was empty, and the balance was set to break contact when 0·5 g of eluate had been collected. This setting does not cause overheating of the motor solenoid coil during operation. The mercury switch and siphon assembly are removed when the time controller is in use. In the last position for collection is fitted a bottomless tube, so that excess of eluate can be collected in a beaker placed under the turn-table.

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A SIMPLE LOCK FOR USE ON A HIGH-VACUUM APPARATUS

The determination of gases in metals by the vacuum fusion technique requires the introduction of the material to be analysed into a high-vacuum apparatus. Samples can be either loaded into an apparatus before outgassing or introduced during a run via a vacuum lock or, in certain special instances, by means of a mercury lift. The first of these approaches has been widely used, and samples can be loaded into a suitably designed manifold or "tree" and released in turn by raising retaining plungers magnetically. "Trees" of this type have been described by Booth, Bryant and Parker[1] and Swann and Williams[2]; Gregory, Mapper and Woodward[3] have given details of a manifold containing hinged trip-buckets operated by solenoids. However, this method of introduction lacks flexibility, and it is often desirable when analysing material having an

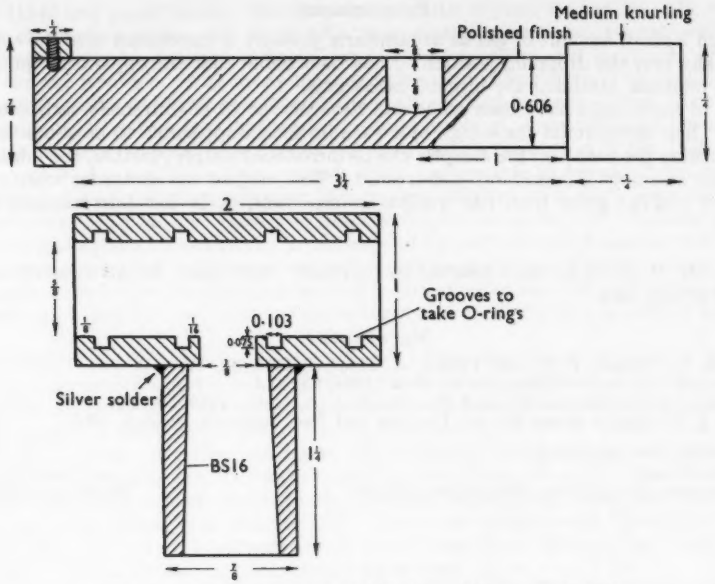

Fig. 1. Construction of lock (all dimensions are in inches)

unknown content of gas to be able to adjust the weight taken after an initial analysis has been carried out. Further, if a "tree" is used, the samples will be stored under vacuum for several hours while the crucible is being outgassed, and for certain types of material this may be undesirable. A suitable vacuum lock can overcome these limitations, as, when such a device is used, samples can be rapidly introduced into the apparatus during an analysis. Although a small vacuum lock has been described recently by Still,[4] it was thought that there was a need for a lock of simple design, which could be easily fabricated.

Design of lock

The lock shown in Figs. 1 and 2 is made of brass or stainless steel and consists of a hollow cylinder, grooved to take two pairs of O-rings (OS12; inside diameter $\frac{5}{8}$ inch, outside diameter

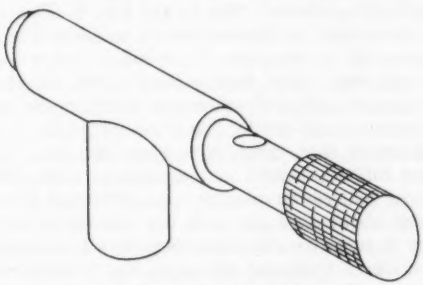

Fig. 2. Assembled lock

$\frac{13}{16}$ inch), through which slides a smoothly polished shaft. The O-rings, which are lubricated with Apiezon L grease, form vacuum seals between the shaft and the cylinder. When the shaft is pushed into the cylinder, the air trapped in the small well containing the sample is pumped rapidly away through the apparatus, and, after a satisfactory working pressure has been attained, rotation of the shaft through 180° allows the sample to drop through the taper joint and thence via a funnel into the crucible.

PERFORMANCE

Although a small amount of gas at atmospheric pressure is introduced into the system when the shaft is slid into the dropping position, it has been found that the apparatus "pumps down" after 4 to 5 minutes, attaining the original blank rate.

Several of these locks have been made and have proved to be extremely satisfactory during operation. Their use permits the design of a vacuum fusion apparatus to be simplified, as both the metal forming the bath and the samples can be introduced simply; further, the "dead" volume of the furnace assembly is considerably decreased. This may be advantageous when a high rate of transfer of evolved gases from the crucible is important, as in the determination of oxygen in beryllium.

I thank Mr. W. R. Johnson, Chemical Inspectorate, War Office, for his valuable help in the construction of this lock.

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Received *April 13th*, 1961

Book Reviews

THE COMPOSITION OF FOODS. By R. A. MCCANCE, C.B.E., M.D., Ph.D., F.R.C.P., F.R.S., and E. M. WIDDOWSON, D.Sc. Medical Research Council Special Report Series No. 297 (Third revised edition of Special Report No. 235). Pp. viii + 252. London: Her Majesty's Stationery Office. 1960. Price 30s.

Having, scientifically speaking, grown up with a book as with "McCance and Widdowson" and confronted with a new edition, one has got about an equal chance of being blind to its faults and unappreciative of its virtues. Which of the two looms the larger probably depends on the mood of the reviewer at the "moment of truth," which is doubtless the moment when he first puts pen to paper or finger to key.

So I have perhaps been wise to hesitate for some weeks before venturing to express in print any opinion on this, the third revised edition of the Medical Research Council's Special Report No. 235, now renumbered as shown above. The pause has, I hope, given time for enthusiasm and wonder to be replaced by critical evaluation and a sense of historical perspective. But it is indeed difficult, if not impossible, to recapture the mood in which we welcomed the first paper-bound edition in the grim year 1940. *The Analyst* itself (1940, **65**, 458) records the birth of the Special Report in a purely formal (unsigned) summary, which opens with the reminder that the Report "supplements, but does not supersede, the three Reports . . . previously issued by the Council." A little research shows that these three early Reports, the scaffolding amid which the present noble edifice was built, appeared as long ago as 1929, 1933 and 1936. It is more difficult to think back to those still earlier pioneer days when our knowledge about the detailed nutrient analysis of foods was about on a par with our knowledge about nutrient requirements and both were so elementary as to be an embarrassment to all concerned.

It is more due to the work of Professor McCance, Dr. Widdowson and their succession of colleagues at Cambridge than to that of any other worker or group of workers, living or dead, that to-day our knowledge of the former, even when only to be given as a mean or modal value within a pretty wide range, has outstripped our information about the latter, which we can still express as no more than a figure, and an average one at that, for the mythical "normal" man, woman or child. Still, it's good to find even an islet of *terra firma* to sustain the increasing pressure of the questing nutritionist.

To describe in detail how the 252 pages of the new (cloth-bound) Report No. 297 differ from the 156 pages of the previous (1946 and cloth-bound) Report No. 235, and they in turn from

the earlier (1942 and paper-bound) third impression of the original Report No. 235, would surely be a work of supererogation. All those who have to-day a dog's-eared copy—and all extant copies must assuredly be in that condition—of any earlier edition will inevitably order and immediately use the new one. And many new arrivals at the camps of food technology, nutrition, dietetics and curative and preventive medicine will meanwhile have joined their ranks. All will require the book, so that none of them will require words of commendation from any reviewer. Even though its price be five times that of the 1946 and 7½ times that of the 1942 edition, and whatever number of it may have been printed, which I am unable to decipher from the Stationery Office's coding, I'll lay all the equipment of a modern kitchen to a single antiscorbutic orange that supplies will soon be exhausted and another impression called for. Scientists had better get in before bibliophiles reduce stocks in the hope of having unearthed another collector's piece. Anyhow, out of sympathy and astonished admiration for the years of perspicacious, painstaking and immaculate work that the authors have had to put into this steadily growing mass of essential data, I trust that they will not be called on to supply another wholly new edition for some years to come.

Meanwhile, it should suffice to say that Part I of the new Report adheres to the admirable pattern and conventions adopted before, but that it now includes two further Parts—a short one on the amino acid make-up of the proteins in all important animal and vegetable foods containing significant quantities and a longer one on the vitamin contents of all our main foods. The former covers 18 amino acids, including the eight—or perhaps it is ten—"essential" ones: the latter gives figures, when appropriate, for carotene and the fat-soluble vitamins A and D and for all the main water-soluble vitamins of the "B group," including vitamin B_{12}, which they do not call by its official name of cobalamin, and ascorbic acid. And I am glad to add, though not in the least surprised to find, that they steadfastly refuse to call riboflavin "vitamin B_2," which it isn't, or nicotinic acid "niacin," which it never should have been.

Perhaps a slightly querulous question may here be addressed to the Publication Section of the M.R.C.—or should it perhaps be to H.M. Stationery Office? When can the last two letters of the word "gramme" be finally, formally and officially dropped in scientific British literature? Even before we all go over to the metric system, this could save an appreciable amount of space.

As heretofore, all that the authors have to say in their General Introduction, and now what they add in the special Introductions to the three Parts of the book, along with their numerous *obiter dicta*, help to make this Report something far more than might have been expected from its original purpose as a catalogue of analytical results presented as nutrient "data." It is indeed a book without peer or parallel, especially for those working in relevant fields in the United Kingdom and the Commonwealth, but also elsewhere. Few, if any, of them can do without it for a day unless they are prepared to limit their efforts by a self-inflicted handicap. A. L. BACHARACH

HANDBUCH FÜR DAS EISENHÜTTENLABORATORIUM. Band I. DIE UNTERSUCHUNG DER NICHTMETALLISCHEN STOFFE. Edited by the Chemikerausschuss des Vereins Deutscher Eisenhüttenleute. Second Edition. Pp. xviii + 322. Dusseldorf: Verlag Stahleisen M.B.H. 1960. Price DM43.

The Chemist's Committee of the Vereins Deutscher Eisenhüttenleute, which corresponds roughly to the Methods of Analysis Committee of B.I.S.R.A., but seems to have somewhat wider terms of reference, has published a four-volume work intended to cover the entire analytical needs of an integrated iron and steel works. This is the second edition of volume I and deals with the analysis of non-metallic materials.

After an introduction explaining the purpose and scope of the work, there follow sections on the analysis of ores, phosphates, slag-forming materials, flue-dust and blast furnace potassium cyanide, slags, cements, refractories, solid fuels, gases, tar, pitch and benzol, fuel oil and lubricants, insulating oil and water. There is no section on sampling in the second edition, as this is now dealt with in volume III, "Probenahme."

Although classical methods form the basis of the work, use is made, when appropriate, of instrumental methods, *e.g.*, photometry, polarography, and potentiometry, but, as explained in the introduction, in order to save space no directions are given on the selection and use of particular instruments. Colorimetric methods indeed are pushed to the limit; the molybdenum-blue method is taken to over 20 per cent. of silica, and the 1,10-phenanthroline method to 60 per cent. of iron, at which level its error is stated to be ±0·20 per cent. Alternative methods are given for most determinations, and the time for an analysis, as well as the probable error, is stated. The latter

is taken as being approximately twice the standard deviation. Here, the German Committee are somewhat ahead of B.I.S.R.A., who have only just begun to think of reproducibility in statistical terms.

In the section on ores, the methods given are general ones, and it may be for this reason that, *e.g.*, the clumsy mercurous nitrate method is retained for determining tungsten. The use of titanous chloride to hasten the reduction of tungsten in its colorimetric determination (British Standard 1121 : Part 32 : 1954) seems not yet to have spread to the Continent. In view of the evident belief of the Committee in potentiometric methods, recommended for chromium, vanadium, antimony and arsenic, it is rather surprising to find no mention of such a method for manganese.

Among the procedures given for refractories are a number taking as long as 60 hours. It is here that one feels that more use could have been made of rapid procedures, such as those published by our own Ceramic Research Association. Neither here nor elsewhere is any mention made of spectrographic methods, although the volume "Probenahme" deals with sampling for spectrographic analysis.

In the section on fuels, oils and greases, where methods tend to be more empirical, extensive reference is made to the methods of the German Institute of Standards (DIN). In all, over thirty existing standard methods are referred to in this work (which is printed according to DIN B5).

In recommending a book of this kind to chemists in British iron and steel works, one has to consider how strong and relatively local are traditions in this field. Nevertheless, although the methods selected would not necessarily be the first choice in a British laboratory, they are undoubtedly well-tried and reliable, and I do not know of any work in English that covers the same field so thoroughly. The method of presentation is uniformly clear and the production and printing are of a high standard. The list of contents at the beginning is so full that anyone using the book would have little need of the index, especially as there is a neat system of cross-references by means of hieroglyphs. Like earlier volumes of the "Handbuch," this one is reasonably priced.

G. M. HOLMES

DIE FLAMMENSPEKTRALANALYSE: GRUNDLAGEN UND VERFAHREN VON FLAMMENPHOTOMETRIE UND FLAMMENSPEKTROGRAPHIE. By Prof. Dr. WOLFGANG SCHUHKNECHT. Pp. xii + 258. Stuttgart: Ferdinand Enke Verlag. 1960. Price (paper) DM 65.50; (cloth boards) DM 69.

The author of volume 48 in the series "Die chemische Analyse" was one of the earliest users of direct-reading flame methods and is therefore well qualified to discuss flame-emission spectroscopy, a technique that is still developing and rapidly extending its field. This is a rather elementary book and deals with the application of flame methods to practical analytical problems; it is directed to the student rather than to the research worker. There is a tendency for equipment of non-German origin to be discussed only when no corresponding German apparatus is commercially available. Thus, all the filter-type flame photometers listed are of German manufacture, whereas almost all the prism or grating instruments mentioned are of non-German origin. The reader is therefore left with a rather unbalanced picture of the equipment currently available.

Professor Schuhknecht restricts the term "flame photometry" to filter instruments and uses "flame spectrophotometry" for prism or grating equipment, a distinction with which not all will agree and which seems out of line with common usage. It is claimed, too, that equipment of the latter type is less convenient to use, again an opinion that will not be universally accepted.

The book is divided into four sections. Section I, discussing the flame as a light source, deals briefly with practical aspects of burner design, gas supply , flame excitation, sample introduction, interference effects and flame-background factors. Section II, on flame photometry and flame spectrophotometry, gives a simple discussion of filters and photocells and details a few commercially available instruments. Atomic-absorption methods are treated in less than two pages, and this section concludes with a consideration of the performance of filter and dispersion instruments from the point of view of interference and other factors of practical importance. Section III deals in 75 pages with laboratory instructions for the application of flame photometry to various types of material. These instructions are much more detailed than is justified in a book of this nature, including, for instance, the preparation of straightforward standard solutions for each of the nineteen methods described for determining alkalis and alkaline earths in chemicals, minerals and biological and agricultural materials. Section IV deals very briefly with flame spectrography.

A bibliography of 496 items, with titles, is much less useful than it might have been, because it is set out in a manner convenient only to the printer. References are arranged by year, alphabetically by author's name within each year. They are printed running on and referred to in

the text by a serial number. The accuracy of the references has not been fully checked, but the page number was omitted from the first one I consulted.

This book, within its limited field, will serve a useful purpose, but there have in the past few years been several more comprehensive and more fundamental monographs on flame photometry.

R. L. MITCHELL

CONTRIBUTI TEORICI E SPERIMENTALI DI POLAROGRAFIA. Volume V. Supplemento a "La Ricerca Scientifica." Pp. 315. Padova, Italy: Centro di Studio per la Polarografia del C.N.R. 1960. Price 2500 Lire.

This supplementary volume of *La Ricerca Scientifica*, which is similar in format to volume IV (see *Analyst*, 1960, **85**, 159), contains the proceedings of two symposia organised jointly by the Italian Polarographic Research Centre and the University of Padua and held at Bressanone in August, 1959. The 12 papers (all in Italian) contributed to the first symposium on the correlation of chemical constitution with physical properties are largely theoretical in nature, but cover a wide range of physical techniques, including dipole moments and ultra-violet spectroscopy. Those (6 in English, 2 in Italian and 1 each in French and German) submitted to the second symposium, at which relationships between polarographic constants and molecular structure were discussed, are by well known polarographers, such as Elving, Randles, Semerano and Zuman, and will interest analysts concerned with the polarography of organic substances.

J. E. PAGE

SCIENTIFIC RUSSIAN: A TEXTBOOK FOR CLASSES AND SELF-STUDY. By JAMES W. PERRY. Second Edition. Pp. xxviii + 565. New York and London: Interscience Publishers Inc. 1961. Price $9 50; 72s.

Until comparatively recently, few people were aware of the enormous output of research by Russian scientists during the post-war years, and there was a general tendency to underrate their scientific achievements and progress. The increasing number of cover-to-cover translations of Russian scientific journals has done much to alter this situation, but the serious research worker will increasingly experience the need to read papers and books in the original. Indeed, an ability to translate Russian with reasonable facility is becoming as important as the ability to read German. For those, too, who believe that the study of a foreign language is in itself a valuable intellectual discipline, Russian has many features that make it a most attractive alternative to Latin or Greek. But, whatever may be the reason for a student commencing a serious study of Russian, he could find no sounder guide than Perry's excellent book.

Although great experience and care has gone into planning the order of presentation, the treatment is never superficial and the book makes no concessions to the dilettante; although it is broken up into a series of relatively short lessons, subdivided into paragraphs admirably adapted to short spells of spare-time study, industry and application are still essential. Starting with lists of foreign technical terms that have been taken over substantially unchanged into the Russian language, the student is quickly familiarised with the Russian alphabet. At this stage the association of each Russian word with its transliteration (following the system of letter equivalents standardised in *Chemical Abstracts*) is most helpful. Throughout the book all words are shown with their accents, which enables the student to associate it with its sound—a powerful method of memorisation. Paradoxically, this becomes of great value when one is confronted with the typical Russian text-book or paper from which all such accents have been omitted by the printer! Every aspect of the Russian language—so far as it concerns the problems of translation from technical literature—is dealt with in the forty-odd lessons that follow, each of which contains reading and translation exercises. The book concludes with a good index and an extensive Russian - English vocabulary of over 5000 words.

For those who learnt their Russian from the first (1950) edition of this book, the most striking improvement in the second edition will appear in the excellent lay-out achieved by the English firm of printers and in the new typography, particularly in the Russian portion of the text. This has made it possible to decrease the number of pages from 816 to 565, despite the increased number of notes and cross-references, and yet there is an outstanding increase in legibility. Misprints from the first edition appear all to have been corrected, and the more topical reading exercises on Atomic Energy and Interplanetary Travel have been revised. Whether for class work or for independent study, this text is hard to beat, and the price is in no way excessive.

H. IRVING

Publications Received

DETERMINATION OF CHLORIDES. By T. A. STRIVENS, B.Sc. Pp. viii + 55. Cambridge, England: W. G. Pye & Co. Ltd. 1961. Price 10s. 6d.
One of a series of Handbooks of Electroanalytical Methods obtainable from the publisher at York Street, Cambridge, England.

REAGENT CHEMICALS: AMERICAN CHEMICAL SOCIETY SPECIFICATIONS, 1960. Prepared by the 1960 Committee on Analytical Reagents (W. STANLEY CLABAUGH, Chairman). Pp. xvi + 564. Washington, D.C.: Applied Publications, American Chemical Society. 1961. Price $10.00.

NAME REACTIONS IN ORGANIC CHEMISTRY. By ALEXANDER R. SURREY. Second Edition. Pp. x + 278. New York and London: Academic Press Inc. 1961. Price $8.00; 64s.

REFERENCE ELECTRODES: THEORY AND PRACTICE. Edited by DAVID J. G. IVES and GEORGE J. JANZ. Pp. xii + 651. New York and London: Academic Press Inc. 1961. Price $20.00.

THE RADIOCHEMISTRY OF SELENIUM. By G. W. LEDDICOTTE. Pp. vi + 38. Washington, D.C.: National Academy of Sciences—National Research Council. 1961. Price 50 cents.
Nuclear Science Series: NAS–NS–3030.

THE RADIOCHEMISTRY OF THE TRANSCURIUM ELEMENTS. By G. H. HIGGINS. Pp. vi + 35. Washington, D.C.: National Academy of Sciences—National Research Council. 1960. Price 50 cents.
Nuclear Science Series: NAS–NS–3031.

AUSTRALIAN JOURNAL OF EXPERIMENTAL AGRICULTURE AND ANIMAL HUSBANDRY. Vol. I, No. 1, May, 1961. Edited by R. N. SANDIFORD. Pp. 55. East Melbourne, Victoria: Australian Institute of Agricultural Science. Annual Subscription (4 parts per year) £5 (Aust.).
A new journal; Postal Address: 226 Clarendon Street, East Melbourne, C.2, Victoria.

THE RADIOCHEMISTRY OF ALUMINIUM AND GALLIUM. By JOHN E. LEWIS. Pp. vi + 48. Washington, D.C.: National Academy of Sciences—National Research Council. 1961. Price 50 cents.
Nuclear Science Series: NAS–NS–3032.

REPRINTS OF "COULOMETRIC METHODS IN ANALYSIS" BY D. T. LEWIS

REPRINTS of the Review Paper, "Coulometric Methods in Analysis" by D. T. Lewis, published in this issue of *The Analyst* (pp. 494–506), will be available shortly from the Assistant Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, at 5s. per copy, post free. A remittance for the correct amount, made out to The Society for Analytical Chemistry, MUST accompany the order; these reprints are not obtainable through Trade Agents.

Methods for the Analysis of Non-soapy Detergent (NSD) Products

By G. F. Longman and J. Hilton

Society for Analytical Chemistry Monograph No. 1

THE first of the Society's Monographs is being published simultaneously with this issue of *The Analyst*. It is "Methods for the Analysis of Non-soapy Detergent (NSD) Products," by G. F. Longman and J. Hilton, of Unilever Research Laboratory, Port Sunlight, Cheshire, and is an expanded version of the paper delivered by G. F. Longman to the North of England Section in Manchester on Saturday, March 12th, 1960. The Monograph is bound in stout paper covers, uniform in size with *The Analyst*, and contains in 30 pages a complete practical scheme for the determination of all components of most non-soapy detergents at present marketed.